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Reduction of greenhouse gas emission by diet manipulation in swine

by

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ABSTRACT

The quantity of nutrients and greenhouse gases emitted from pig production into the environment can be modulated by different nutritional strategies, but their practical application is dependent upon cost and other biological limitations. This research was conducted to evaluate the nutritional interventions most promising to reduce nutrient excretion and greenhouse gas emissions by pigs. The studies concentrated on sows and growing-finishing pigs, which produce two-thirds of the greenhouse gas emissions by pigs in Canada. The studies evaluated the effect of protein and energy source and protein intake on CO_2 and CH_4 emission and nitrogen and carbon excretion and assessed the effect of high- (HP) and low-protein (LP) diets based on corn-soybean (CS) or wheat-barley-canola (WBC) meal. Also, a study was conducted to evaluate the effect of a combined low-protein, low-phosphorus diet supplemented with limiting amino acids and phytase and xylanase, individually or combination, on nutrient digestibility and energy metabolism in growing-finishing pigs using indirect calorimetry.

LP diets led to a significant reduction in CH_4 production from WBC diet, but emissions were not affected by protein level in the corn diets. The CO_2-equivalents emitted by non-pregnant sows fed at maintenance was lower for the WBC-LP than the WBC-HP diet, while the protein reduction had no effect for the CS diets. Overall, the CO_2-equivalents were reduced by 16.4% by reducing dietary protein contents. The gestating and lactating sow data showed that LP diets supplemented with limiting amino acids significantly reduced N and C excretion (Trial 1). From trial 2, average daily gain was not affected by diet type or protein level. N retention was similar for WBC-LP and WBC-HP, but lower for CS-LP than CS-HP. WBC-LP had non-significantly similar fecal N, but a significantly lower urinary N than WBC-HP. N excretion for CS-LP and CS-HP were similar. CO_2-equivalent (CO_2 and CH_4) emission by pigs was numerically lower for LP than HP diets of CS and WBC. Carbon excretion was lower for CS than WBC diets, but similar for LP and HP diets. Daily gain, daily feed intake and gain to feed were not affected by protein level. O_2 consumption and CO_2 emission were significantly influenced by dietary protein. Dietary protein reduction reduced CH_4 emission numerically. Heat production was less in pigs fed VLP than HP (Trial 3). Similarly, in

trial 4, daily gain, feed intake, gain to feed, O_2 consumption, CO_2 and CH_4 emission, and nitrogen retention were not affected by combined protein and phosphorus reduction. N intake was significantly lower in the protein-reduced diets compared to control. Fecal and urinary N were significantly lower for pigs fed reduced protein diets compared to the control. Heat production non-significantly affected by dietary treatments.

Reducing dietary protein concentration maintained animal performance and reduced nutrient excretion by growing-finishing pigs fed WBC. LP reduced CH_4 emission, but had little effect on CO_2 emission. The CO_2-equivalent arising from the animals (CO_2 and CH_4) tended to be lower for LP. VLP improved heat production, an indication of improved nutrient utilization, and potential for reduced greenhouse gas emission. Such feeding regimens offer substantial benefits in maintaining sustainable environmentally-friendly pork production. Understanding the effect of synthetic AA and exogenous enzyme supplementation on utilization of nutrients (e.g., N and P) and energy will not only encourage swine producers and feed manufacturers to use these nutritional interventions, but also challenge the feed industry to reconsider their diet formulation practices to reduce environmental impact of pork production.

To Grace, Elijah and Emmanuel

To my family and friends who have provided me with continual support and encouragement throughout my educational endeavours.

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TABLE OF CONTENTS

1. INTRODUCTION 1

2. REVIEW OF LITERATURE 2

2.1. Metabolism and Partitioning of Energy in the Body 3

2.1.1. Effect of Digestibility and Metabolizability 8

2.2. Quantitative Determination of Heat Production (HE) 10

2.2.1. Other Methods for Determination of Heat Production (HE) 10

2.3. Supplementation of Synthetic Amino Acids to Swine Diets 12

2.3.1. Commonly Used Synthetic Amino Acids 13

2.4. Low Protein Amino Acid Supplemented Diets for Swine 14

2.5. Potential to Enhance the Nutritional Value of Feedingstuff and Diets 15

2.6. Use of Exogenous Enzymes in Livestock Industry 16

2.6.1. Carbohydrase Supplementation of Livestock Diets 16

2.6.2. Carbohydrase Supplementation of Swine Diets 17

2.7. Phytate and Action of Phytase 18

2.7.1. Effect of Phytase on Energy Metabolism in Swine 19

2.7.2. Effect of Phytase on Protein and Amino Acids Metabolism in Swine 21

2.8. Non-starch Polysaccharides and Action of Xylanase 23

2.8.1. Xylanase Supplementation of Swine Diets 25

2.8.1.1. Effect of Xylanase on Energy Digestibility 26

2.8.1.2. Effect of Xylanase on Protein and Amino Acids Digestibility 27

2.8.1.3. Effect of Xylanase on Dry Matter Digestibility 29

2.8.1.4. Effect of Xylanase on Animal Performance 30

2.9. Potential Applications and Implications of Research on Energy and Protein Metabolism 31

2.10. Summary 35

2.11. Hypotheses and Objectives 36

2.12. Literature Cited 38

3. EFFECT OF LOW PROTEIN DIETS FED TO SOWS AT DIFFERENT STAGES OF THE REPRODUCTIVE CYCLE ON CARBON AND NITROGEN BALANCE 57
3.1. Introduction 57
3.2. Materials and Methods 58
3.2.1. Experimental Design 58
3.2.2. Diet Composition 59
3.2.3. Housing and Feeding 60
3.2.4. Growth Measurements 61
3.2.5. Respiration Measurements 61
3.2.6. Chemical Analyses 63
3.2.7. Calculations 63
3.2.8. Statistical Analyses 64
3.3. Results 64
3.3.1. CO_2 Emission by Gestating and Lactating Sows 66
3.3.2. GHG Emission by Sows 67
3.4. Discussion 68
3.5. Conclusion 79
3.6. Implications 80
3.7. Literature Cited 81

4. EFFECT OF PROTEIN AND ENERGY SOURCE AND PROTEIN INTAKE ON PERFORMANCE, NUTRIENT EXCRETION AND GREENHOUSE GAS EMISSION BY GROWING-FINISHING PIGS 98
4.1. Introduction 98
4.2. Materials and Methods 100
4.2.1. Experimental Design 100
4.2.2. Diet Composition 101
4.2.3. Housing and Feeding 101
4.2.4. Growth Measurements 102
4.2.5. Respiration Measurements 102

4.2.6. Chemical Analyses 103
4.2.7. Calculations 104
4.2.8. Statistical Analyses 106
4.3. Results 107
4.3.1. Animal Performance 107
4.3.2. Nitrogen Balance 108
4.3.3. Nutrient Excretion (N and C) 108
4.3.4. Oxygen Consumption, CO_2 and CH_4 Emission 109
4.3.5. Gas Exchange and GHG Emission 109
4.4. Discussion 109
4.5 Conclusion 112
4.6. Implications 113
4.7. Literature Cited 114

5. EFFECTS OF VERY LOW PROTEIN DIETS FED TO GROWING-FINISHING PIGS ON PERFORMANCE AND GREENHOUSE GAS EMISSION 128
5.1. Introduction 128
5.2. Materials and Methods 130
5.2.1. Experimental Design 130
5.2.2. Diet Composition 131
5.2.3. Housing and Feeding 132
5.2.4. Body Weight Measurements 132
5.2.5. Respiration Measurements 133
5.2.6. Chemical Analyses 133
5.2.7. Calculations 134
5.2.8. Statistical Analyses 137
5.3. Results 137
5.3.1. Animal Performance 137
5.3.2. Oxygen Consumption, and CO_2 and CH_4 Emission 138
5.3.3 Energy Metabolism 138

5.3.4. Nutrient Intake and Excretion (N and C) 138
5.3.5 Gas Exchange and GHG Emission 139
5.4. Discussion 139
5.5. Conclusion 147
5.6. Implications 147
5.7. Literature Cited 149

6. EFFECT OF PHYTASE-XYLANASE ADDITION TO WHEAT-BASED DIETS ON PROTEIN AND ENERGY METABOLISM IN GROWING-FINISHING PIGS FED AD LIBITUM 168
6.1. Introduction 168
6.2. Materials and Methods 170
6.2.1. Enzymes 171
6.2.2. Experimental Design 171
6.2.3. Diet Composition 172
6.2.4. Housing and Feeding 12
6.2.5. Body Weight Measurements 173
6.2.6. Nitrogen and Energy Balance 173
6.2.7. Respiration Measurements 174
6.2.8. Chemical Analyses 175
6.2.9. Calculations 175
6.2.10. Statistical Analyses 178
6.3. Results 179
6.3.1. Dietary Protein Reduction 180
6.3.2. Phosphorus Reduction 181
6.3.3. Enzyme Supplementation 182
6.3.3.1. Phytase Addition 182
6.3.3.2. Phytase-Xylanase Interaction 183
6.3.3.3. Xylanase Addition 184
6.4. Discussion 185
6.5. Conclusion 193

6.6. Implications 194
6.7. Literature Cited 195

7. SUMMARY, GENERAL DISCUSSION AND FUTURE DIRECTIONS 218
7.1. Prospects for Future Research 223
7.2. Literature Cited 226

LIST OF TABLES

Table 3.1a: Ingredient (g/kg) composition of diets fed to non-pregnant sows (as fed basis) 83

Table 3.1b: Ingredient (g/kg) composition of diets fed to gestating sows (as fed basis) 84

Table 3.1c: Ingredient (g/kg) composition of diets fed to lactating sows (as fed basis) 85

Table 3.2a: Analyzed (g/kg) nutrient content of diets fed to non-pregnant sows 86

Table 3.2b: Analyzed (g/kg) nutrient content of diets fed to gestating and lactating sows 87

Table 3.3a: Feed, protein and carbon intake (g/d) of non-pregnant sows fed high or low protein diets based on barley or corn 88

Table 3.3b: Feed intake (kg/d) and carbon intake (g/d) of high or low protein diets during two consecutive parities 89

Table 3. 4: Carbon dioxide and methane emission and carbon dioxide equivalents (g/d) produced by non-pregnant sows fed diets at two protein levels based on barley or corn 90

Table 3.5: Carbon dioxide and methane emission (g/d) from non-pregnant sows fed high or low protein diets based on barley or corn 91

Table 3.6: Carbon dioxide equivalents (g/d) per non-pregnant sows fed high or low protein diets based on barley or corn 92

Table 3.7a: Carbon and nitrogen excretion (g/d) during gestation and lactation in sows fed high or low protein diets based on barley or corn 93

Table 3.7b: Effect of diet, stage and parity on CO_2 emission (g/d) from sows during gestation and lactation 94

Table 3.8: Carbon dioxide equivalents (g/d) during gestation and lactation in sows fed high or low protein diets based on barley or corn 95

Table 3.9: CO_2-equivalents produced by one sow per year when fed conventional or reduced protein diet 96

Table 4.1: Ingredient (g/kg) composition of experimental diets 120

Table 4.2: Analyzed (g/kg) nutrient composition of diets 121

Table 4.3: Performance and nutrient retention of growing-finishing pigs fed conventional or reduced protein diets based on wheat-barley or corn 122
Table 4.4: Nitrogen and carbon excretion (g/d) in feces and urine of growing-finishing pigs fed conventional or reduced protein diets based on wheat-barley-canola or corn-soybean meal 123
Table 4.5: Relationship between 4 and 24 h gas exchange measurements in growing-finishing pigs fed corn-soybean meal diets 124
Table 4.6: Gas exchange and greenhouse gas emission (g/d) of growing-finishing pigs fed conventional or reduced protein diets based on wheat-barley or corn 125
Table 5.1: Effect of replacing one gram protein by one gram starch 157
Table 5.2: Ingredient (kg/tonne) composition of control (HP) and very-reduced protein, amino acid supplemented (VLP) experimental diets 158
Table 5.3: Calculated (g/kg) nutrient composition of experimental diets 159
Table 5.4: Analyzed (g/kg) nutrient composition of experimental diets 160
Table 5.5: Effect of very-reduced protein, amino acid supplemented diet on performance of growing-finishing pigs fed *ad libitum* 161
Table 5.6: Effect of very-reduced, protein amino acid supplemented diet on gas exchange, carbon dioxide equivalents and heat production of growing-finishing pigs fed *ad libitum* 162
Table 5.7: Effect of very-reduced protein amino acid supplemented diet (g/d) on intake and utilization of N and C in growing-finishing pigs fed ad libitum 163
Table 5.8: Effect of very-reduced protein amino acid supplemented diet on gas exchange (L/d), CO_2-equivalent (kg/d) and heat production (kJ/min) of growing-finishing pigs fed ad libitum 164
Table 5.9: Suggested respiratory quotients (RQ) to be used for cattle, pigs and poultry 165
Table 6.1: Ingredient and nutrient (g/kg) composition of diets 207
Table 6.2: Analyzed (g/kg) nutrient composition of experimental diets 208
Table 6.3: Mean chemical composition (%) of feces and urine samples from growing-finishing pigs fed wheat-based diets *ad libitum* 209

Table 6.4: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on growth performance in growing-finishing pigs fed wheat-based diets *ad libitum* 210

Table 6.5: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on nitrogen balance in growing-finishing pigs fed wheat-based diets *ad libitum* 211

Table 6.6: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on parameters of carbon balance in growing-finishing pigs fed wheat-based diets *ad libitum* 212

Table 6.7: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on energy metabolism in growing-finishing pigs fed wheat-based diets *ad libitum* 213

Table 6.8: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on intake of DE and ME (MJ/kg) and digestibility (%) of nutrients in growing-finishing pigs fed wheat-based diets *ad libitum* 214

Table 6.9: Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on parameters of energy flow (MJ/kg) in growing-finishing pigs fed wheat-based diets *ad libitum* 215

Table 6.10: Effects of dietary protein and phosphorus level on energy losses (%) and efficiency of nutrient and energy utilization in growing-finishing pigs fed wheat-based diets *ad libitum* 216

Table 6.11: Effects of dietary protein and phosphorus level, xylanase, phytase, and combined xylanase-phytase on O_2 consumption (g/d), CO_2 and CH_4 emission (g/d), CO_2-equivalent (g/d), respiration quotient, heat production and heat increment (MJ/d) of growing-finishing pigs fed wheat-based diets *ad libitum* 217

LIST OF FIGURES

Figure 2.1: Energy partitioning in the pig body 4

Figure 2.2: Model of energy flow 5

Figure 3.1: Schematic representation of the experimental design 97

Figure 4.1: Schematic representation of the experimental design for the study 126

Figure 4.2: Timeline representing the housing and feeding regimen during the pre-balance, balance and respiration periods for each block of pigs 127

Figure 5.1: Schematic representation of the experimental design for the study 166

Figure 5.2: Timeline representing the housing and feeding regimen during the pre-balance, balance and respiration periods for each block of pigs 167

LIST OF ABBREVIATIONS

Abbreviations	Definitions
AA	Amino acid(s)
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain(s)
ADL	Acid detergent lignin
ANF	Anti-nutritional factor
ANOVA	Analysis of variance
BW	Body weight
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
CO_2-eq	Carbon dioxide equivalent
CP	Crude protein (N x 6.25)
CS	Corn-soybean meal
d	day(s)
DCP	dicalcium phosphate
DE	Digestible energy
DM	Dry Matter
Exp.	Experiment(s)
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
FTU	Phytase units
FXU	Xylanase units
g	gram(s)
GHG	Greenhouse gas
GWP	Global warming potential
h	hour(s)
HE	Heat Energy (or Heat Production)
HP	High protein (conventional or control)
kcal	kilocalorie
kg	kilogram
L	Litre
LP	Low-protein
ME	Metabolisable Energy
meq	milliequivalent
min	minute(s)
mL	milliliter
MCP	Monocalcium phosphate
MJ	megajoules
MW	molecular weight
N	Nitrogen
N	Normal distribution

n	sample size
NDF	Neutral detergent fiber
NE	Net energy
NSP	Non-starch polysaccharide
N_2O	Nitrous oxide
O_2	Oxygen
P	Probability
ppm	parts/million parts
pu	percentage units
r	simple correlation coefficient
RSD	Relative Standard Deviation
SAS®	Statistical Analysis System
SD	Standard deviation
SEM	Standard Error of the Mean
vol	volume
vs.	versus
wk	week(s)
WBC	Wheat-barley-canola meal

1.0 INTRODUCTION

The very inefficient use by swine of total dietary nitrogen (N), carbon (C) and phosphorus (P) poses nutritional and environmental problems (Lenis and Jongbloed, 1999). Supplementation of synthetic or free amino acids to swine diets is a key nutritional strategy to address these problems of excess proteins and amino acids (Möhn and Susenbeth, 1995; Mathison et al., 1999). Synthetic and/or exogenous enzymes can be used to increase N and C digestibility (Bedford and Schulze, 1998). However, their application by the swine industry depends not only on their impact on the environment, but also on their economic benefit and acceptance at the farm gate. Understanding the effect of synthetic and exogenous enzyme supplementation on the utilization of various nutrients (notably nitrogen, non-starch polysaccharides and phosphorus) will not only encourage swine producers and feed manufacturers to use these nutritional interventions, but also challenge the feed industry to reconsider the environmental impact of their diet formulation practices.

The expansion of the pork industry in western Canada has raised the expectation for careful management of excreted nutrients. Nitrogen and phosphorus are the two major nutrients of concern that are excreted in feces and urine, and may have a major environmental impact if not managed properly. Effective nutrient management is the key for sustainable pork production, and dietary manipulation may be effective in reducing N and P excretion by pigs (Lenis and Jongbloed, 1999).

Nutrient digestibility is an important factor affecting overall efficiency of N and P utilization in pigs. Progress in enzyme technology has produced supplemental enzyme cocktails that may assist pigs and poultry to digest less-digestible fractions in the diet (Bedford and Schulze, 1998), thereby increasing nutrient digestibility and lowering feed cost. For example, phytase has consistently increased digestibility of phytate-P in ingredients fed to pigs (Jongbloed et al., 1992). Enzymes that hydrolyze non-starch polysaccharides (NSP) in cereal grains (e.g., β-glucanase and xylanase) have been investigated, but did not improve nutrient digestibility consistently across studies (Bedford and Schulze, 1998).

In a report 'Feeding Strategies for Mitigating Greenhouse Gas Emissions from Domestic Animals' (Mathison et al., 1999) the above approaches (supplementation of synthetic or free amino acids to swine diets and use of exogenous enzymes) were recommended, with reduction of dietary crude protein (CP) identified as the single most effective strategy. However, there is a dearth of information in the literature to support and justify adoption and application of some of the proposals/recommendations in the report. The reduction of nitrogen excretion by the animals may lead to a proportional reduction in nitrous oxide (N_2O) emissions from manure, but this has not been adequately tested. Methane (CH_4) emissions appear linearly and positively related to the intake and digestibility of NSP (Jensen, 1996). However, the cereals on which sow diets are predominantly based (barley in Alberta; corn in Ontario) have not been tested for their effect on CH_4 emissions by swine, most especially sows. Also, it is not known whether there is an influence of diets with low protein content on CH_4 emissions.

A literature review was conducted to assess the current state of knowledge and opportunities for new research. A series of experiments were subsequently designed to address the nutritional intervention strategies most promising to reduce nutrient excretion and greenhouse gas (GHG) emissions by pigs. These studies concentrated on sows and growing-finishing pigs, which produce two-thirds of the GHG emissions by pigs in Canada. The emphasis has been on improving the efficiency of retention of dietary nitrogen and carbon by increasing protein and energy efficiency. This can be achieved by using low protein diets supplemented with synthetic amino acids. Nutritional approaches to reducing emissions of carbon dioxide (CO_2), methane (CH_4) and nitrogen (N) from sows and growing-finishing pigs were also developed.

2.0 REVIEW OF LITERATURE

The report "Feeding Strategies for Mitigating Greenhouse Gas Emissions from Domestic Animals" (Mathison et al., 1999) identified a reduction of dietary protein content in swine rations as the single most effective strategy for reducing CO_2 emissions from pigs. The subsequent reduction in nitrogen excretion may also lead to an equivalent reduction in N_2O emissions from manure (Misselbrook et al., 1998). The effects of dietary protein

reduction on efficiency of protein retention are well documented. For example, Möhn and Susenbeth (1995) showed that a 20% reduction in dietary protein, below current recommendations for growing pigs, could reduce nitrogen excretion by as much as 35%, provided that the low protein diets provide adequate amino acid intake. Similarly, the use of amino acid-supplemented low-protein diets for pregnant sows showed promise (Corley et al., 1983). However, the possible scope for improvement of protein efficiency remains to be determined (Möhn et al., 2000). An additional benefit of reduced dietary protein intake is a reduction in the animals' CO_2 emissions resulting from an improved utilization of dietary energy (Möhn and Susenbeth, 1995). Direct measurements of the effectiveness of reduced dietary protein on CO_2 emissions from swine are currently not available. Similarly, possible effects of a reduction of dietary protein intake on CH_4 emissions have not been studied.

2.1 Metabolism and Partitioning of Energy in the Body

Energy is not a nutrient per se, but a quality associated with the nutrient content of feedstuffs and mixed diets. Carbohydrates – starch, sugar and fibre – serve as substrates for oxidation (the process that convert carbon from the diet to carbon dioxide, CO_2) to provide energy for metabolic processes. Feed protein and lipids in a diet mostly serve as components for body protein and lipid deposition, but can also be oxidized to provide energy for metabolic processes. The amount of energy that can be derived from dietary nutrients differs among protein, lipids and carbohydrates because of different proportions of carbon and hydrogen in their moiety, thus producing different amounts of heat or energy on oxidation.

Common systems to describe dietary energy are gross (GE), digestible (DE), metabolizable (ME) and net (NE). Pigs rarely retain more than 50% of the GE (or heat of combustion) provided. Although for most diets 80 to 90% of GE is digested, not all this energy is available for metabolism because energy will be lost in the urine and as CH_4 and heat. The ME content of a diet is the difference between DE and these "material" energy and endogenous losses. Thus, all ME not retained by the animal is lost as heat. Such components are illustrated in Figures 2.1 and 2.2.

Energy Partitioning in the Pig Body

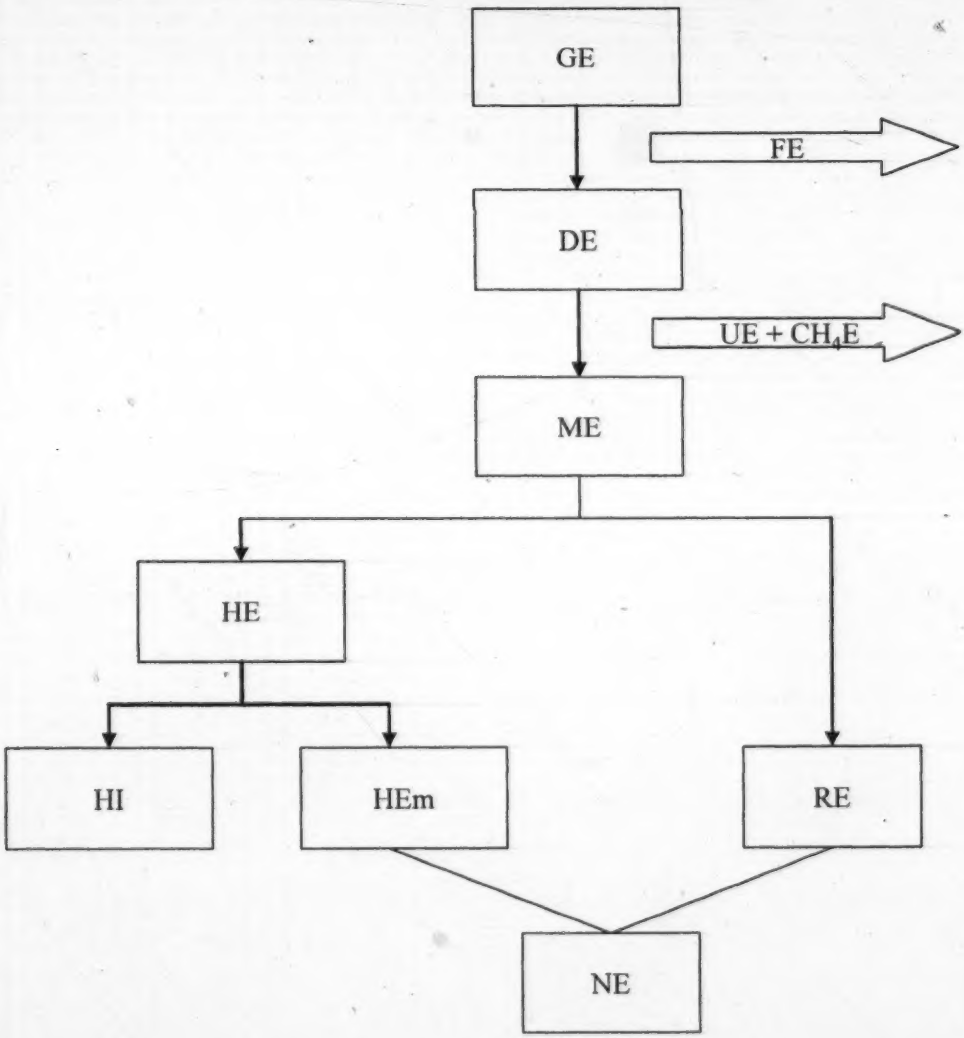

Figure 1.1 Energy partitioning in the pig body. Gross energy (GE), fecal energy (FE), digestible energy (DE), urinary energy (UE), methane energy (CH_4E), metabolizable energy (ME), heat production (HE), heat increment (HI), heat of maintenance (HEm), retained energy (RE), net energy (NE). [Adapted from Chwalibog and Thorbek, 2000]

Model of Energy Flow

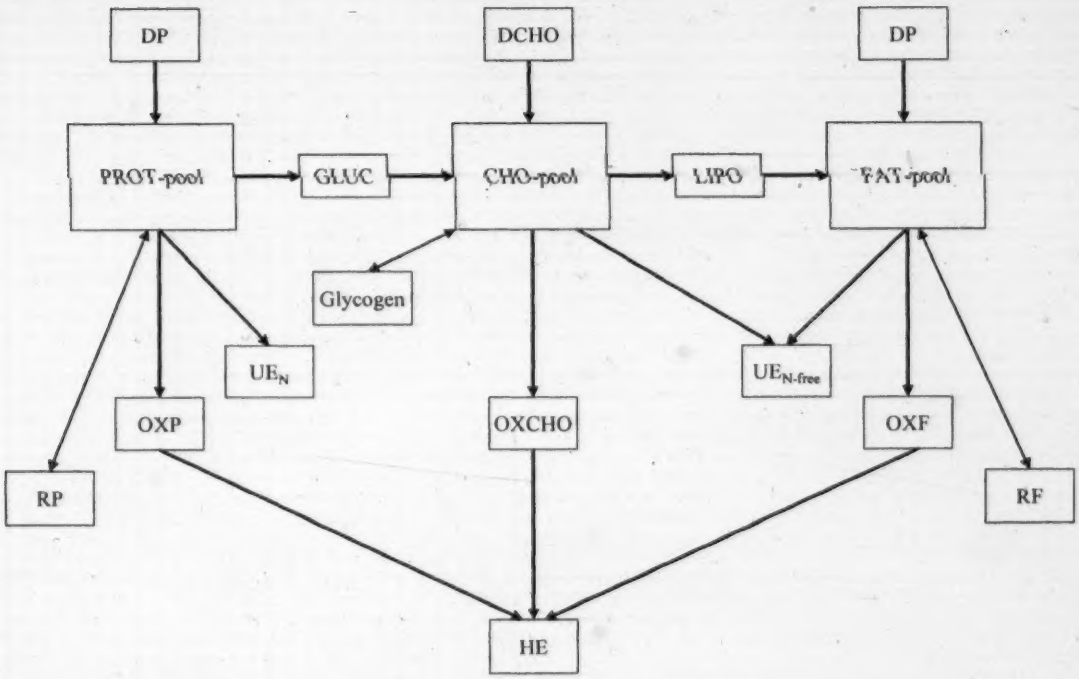

Figure 1.2 Model of energy flow between substrates and products during feeding and non-feeding. Digested protein (DP), protein retained or mobilized (RP), oxidized protein (OXP), urinary energy from nitrogenous components (UE_N), energy transfer from protein to carbohydrate pool (GLUC). Digested carbohydrate (DCHO), carbohydrate retained or mobilized (Glycogen), oxidized carbohydrate (OXCHO), urinary energy from N-free components ($UE_{N\text{-free}}$), energy transfer from carbohydrates to fat pool (LIPO). Digested fat (DF), oxidized fat (OXF), fat retained or mobilized (RF), and total heat production (HE). [Adapted from: Chwalibog et al., 2004]

The retained energy, RE, (primarily protein and lipid) can be measured directly by the comparative or serial slaughter technique, but that lost as heat is not assessed (Quiniou et al., 1995; van Milgen et al., 1997; Birkett and de Lange, 2001). Although comparative or serial slaughter requires simple equipment, it is extremely laborious and gives an estimate

of the average RE over a long period of time (Quiniou et al., 1995 Birkett and de Lange, 2001). Accurate results are only obtained when the number of animals is large enough, so that the inevitable large variation caused by between-animal variation does not have a great effect. This method is very expensive and laborious when used for cattle and pigs, but is often used for chickens (Birkett and de Lange, 2001). Alternatively, RE and ME can be determined by measuring heat production (HE) in respiration studies. Most commonly, indirect calorimetry is used, which is based on the measurement of gas exchanges between the animal and its environment (van Milgen et al., 1997; van Milgen and Noblet, 2000). When nutrients are oxidized, animals consume O_2 and produce CO_2, whereas CH_4 is produced by gut microbes during enteric fermentation (Itabashi et al., 1984; Whitelaw et al., 1984; Wolin and Miller, 1987; Rouviere and Wolfe, 1988). The dynamics of gas exchanges and N excretion from protein catabolism combined with the stoichiometry of nutrient oxidation allow sensitive, reliable and precise calculation of heat energy (Brouwer, 1965).

Both indirect calorimetry and comparative-serial slaughter techniques provide an estimate of the total energy balance of the animal, which in combination with the N and carbon balances, allows calculation of protein and lipid retention. However, calorimetry has the advantage over the comparative or serial slaughter technique in that it can be used to measure energy balance over successive short periods of time, even within days, and different components of HE (van Milgen et al., 1997; van Milgen and Noblet, 2000). The calorimetry technique typically gives higher estimates for energy and protein retention than does the comparative-serial slaughter technique (Quiniou et al., 1995; Birkett and de Lange, 2001b) because the "material" and endogenous losses are well accounted for. The combination of digestibility and respiration (measurement of HE) has the further advantage of being used to derive equations to predict NE content of complete diets (Noblet et al., 2003; CVB, 2003; Nehring and Haelein, 1973; Hoffmann et al., 1993). Such an advantage could be exploited for the introduction of the NE system in Canada, especially in the Prairie region, where swine diet formulation utilizes a wider variety feed ingredients than the simple corn-soybean meal diets commonly used in the US. The main difference between DE or ME and NE is that the former two express "potential" energy,

while the latter expresses metabolically useable energy, and includes the efficiency with which the nutrients can be utilized. This efficiency is different among nutrients.

Body protein is subject to a constant breakdown and synthesis process, during which a certain fraction of amino acids is inevitably lost. Protein turn-over requires energy and the repeated breakdown and synthesis of protein increases this energy expenditure; this means that digested protein is used with a mean efficiency of only 54% for body protein retention (ARC, 1981). In comparison, starch and lipids are utilized for lipid deposition with a mean efficiency of 74% and 90%, respectively (de Lange and Birkett, 2004). During hindgut digestion of fibre, volatile fatty acids (VFA) are produced, which are used with a lower efficiency than sugars absorbed after small intestinal digestion, resulting in an efficiency for lipid deposition of only 50% (van der Meulen et al., 2001).

The ME content in common feedstuffs rich in protein and/or fibre is lower than their corresponding DE content, and the relative ranking of feedstuffs according to their energy content, under the ME and DE system, is similar (NRC, 1998; Rademacher, 2001). However, when using the NE system, the ranking of energy content of feedstuffs rich in protein and/or fibre is considerably lower compared to their energy content under the ME and DE system. For example, soybean meal has a greater DE and ME content than barley, but considerably lower NE content. Canola meal and sunflower meal, which contain large amounts of protein and fibre, are valued much lower relative to barley under the NE system. Conversely, fats and oils are assigned much greater value under NE compared to the DE or ME system.

The energy evaluation system also affects the cost of energy (i.e., dollar/tonne divided by MJ energy) of different feedstuffs. Again, the cost per MJ for protein-rich feedstuffs under the NE system is relatively higher, while the cost/MJ for fat-rich feedstuffs, is relatively lower compared to the DE or ME system. The consequence for diet formulation is that diets formulated under the NE system will be lower in protein (Rademacher, 2001), because the cost/MJ energy inhibits the inclusion of excess protein via large amounts of soybean or canola meal, in favor of the inclusion of free AA. Additionally,

choosing ingredients with a lower cost of net energy will reduce the cost of a mixed diet by as much as 2% during the grower and finisher phases (Rademacher, 2001) compared to formulation using ME or DE. Because of the greater net energy efficiency of the low protein diets, the DE or ME contents of such diets are lower compared to diets with greater protein content. Conversely, the increased value (lower cost/MJ) for fat-rich feedstuffs, when using NE versus ME or DE, allows increasing the net energy of a diet, without a cost penalty for greater inclusion rates of such fat-rich feeds. Increased net energy content of diets is usually beneficial because the growth rate of pigs is often limited by their energy (feed) intake.

2.1.1 Effect of Digestibility and Metabolizability of Feedstuffs on Nutrients

The processes of digestion, absorption, retention and excretion of nutrients in mammals, are made up of several complex processes that may be influenced by a wide range of factors. Studies reported in the literature indicate that the digestibility coefficient of energy (DCe or DE:GE ratio) is affected by factors not related to the diet itself. In growing pigs, DCe increases with body weight (BW) (Noblet and Shi, 1994), with the largest differences occurring for high fibre feeds. The largest effect of BW is observed when adult sows and growing pigs are compared: digestibility coefficients are superior in all cases for the sows, the difference being greatest with fibrous diets or ingredients (Fernandez et al., 1986; Noblet and Shi, 1993). In addition, the difference also depends on the type of fibre. For instance, the values measured for wheat bran and corn gluten feed in growing pigs represented 90 and 70% of the value recorded in sows, respectively (Noblet and Bourdon, 1997).

Techniques employed in nutritional energetics of animals have classically been concerned with the partitioning of dietary energy into faecal, urinary, methane, heat, and recovered or product energy. How well an animal can digest and assimilate nutrients for productive purposes depends upon the bioavailability of the nutrients in the diet, as well as absorption capacity of the digestive tract, efficiency of metabolism and rate of retention (Church and Pond, 1988). However, it is the bioavailability of the nutrient in the diet that would ultimately determine how much of the ingested nutrient would be voided

as feces. Three main factors affect the amount of nutrient losses in feces, namely amount consumed, efficiency of conversion to digestible energy (between animal variations in digestibility) and amount of endogenous secretions that are not reabsorbed. The primary opportunity for reducing the amount of nutrient excreted by animals is to reduce dietary intake but at the same time optimize the efficiency of utilization of ingested nutrients (Paul et al., 1998).

The goal of efficient and productive feeding of animals, within economic and environmental constraints, is to provide essential available nutrients for maintenance and production with minimal excesses and losses. Therefore, any small differences detected in the digestibility or availability of nutrients should be exploited to increase the potential for animal use and reduce the amount of losses in the overall production system (Luiting, 1999).

Two possible sources of variation in feed efficiency between animals may originate from energy losses in the form of heat and gaseous products of fermentation between the conversions of DE to NE. The NE of food is the difference between ME and heat increment due to feeding and fermentation. Heat increment (or specific nutrient dynamic effect) is the heat production associated with nutrient digestion and metabolism over and above that produced prior to ingestion (Church and Pond, 1988).

Several techniques are available for measuring heat production in animals. A direct calorimetry technique involves enclosing an animal in a well-insulated chamber and measuring heat loss by means of thermocouples, circulation of water in pipes in the chamber or by electrical means using gradient layer calorimetry (Church and Pond, 1988). Indirect measurements of heat production are based on the determination of oxygen consumption, carbon dioxide and methane production (Delfino et al., 1988; Young et al., 1984). Several techniques have been developed for estimating heat production from these measurements (McLean and Tobin, 1990).

2.2 Quantitative Determination of Heat Production

The metabolic processes that result in supplying and using energy in the body can be determined by measuring the heat production (HE) of the animal (McLean and Tobin, 1990; Tauson et al., 1994; Tauson et al., 1997, Chwalibog et al., 2004). The quantity of energy produced in the oxidative processes of carbohydrates, fat, protein and short chain fatty acids (SCFA) can be measured as heat (McLean and Tobin, 1990). An animal uses 70% of carbohydrate and fat energy, and 40% of protein energy for energy-requiring processes, whilst the rest is lost as heat. Part of the energy transferred to the energy-rich phosphorus compounds and later hydrolysed will also, sooner or later, be converted to heat (Young et al., 1984; Noblet and Bourdon, 1997). This means that all of the metabolic processes can be quantified on the basis of the heat production of the animal.

2.2.1 Other Methods for Determination of Heat Production

The methods for estimation of HE and RE described above require the use of respiration chambers (Young et al., 1984; McLean and Tobin, 1990; Noblet and Bourdon, 1997), but attempts have been made to measure RE in a simpler way, based on the fact that the weight gain of an animal is the result of retention of water, protein, fat and minerals.

The day to day retention of protein and ash could be determined by balance studies (Chwalibog, 2004; Chwalibog et al., 2004). If the weight of water in the living animal can be measured, the weight of stored fat can be estimated by subtracting the lean body mass. In practice, total body water can be estimated by so-called "dilution" techniques, in which a known quantity of a tracer substance is injected into the animal, allowed to equilibrate with the body water, and its equilibrium concentration determined. To provide valid data, a tracer must equilibrate rapidly and completely in all body water compartments, and must be capable of being measured accurately in body fluids (Chwalibog, 2004). Several substances including antipirine, urea and hydrogen isotopes have been used to measure body water. Hydrogen isotopes have been the most thoroughly researched body water tracers. Both deuterium oxide (D_2O) and tritiated water (TOH) cross barriers at the same rate as water and distribute evenly in body fluids making them ideal tracers. However, there are some drawbacks to this method, firstly

overestimation of body water and secondly the difficulties in quantification of ingested water and that contributed by metabolism.

Direct determination of muscle or body protein using: (a) body potassium measurement (Chinn, 1967), (b) creatinine excretion (Chinn, 1967), (c) neutron activation analyses (Sutcliffe et al., 1992; Hansen et al., 1999; Krishnan and Sturtridge, 1999), and (d) ^{51}Cr-labelled red blood cells (Chwalibog, 2004) have also been evaluated in body composition studies. The most extensive research has been attributed to potassium either by using whole body counting (^{40}K) or isotope dilution (^{42}K). Both techniques are based on the presence of potassium as the major intracellular cation; however, they are expensive and not precise enough. Measurements of creatinine excretion should, in theory, be proportional to muscle mass, but there is a large variation in creatinine excretion both between and within days, resulting in inconsistent results.

Computer tomography (CT) and nuclear magnetic resonance (NMR) have been used to provide cross-sectional images of rabbits, chickens and pigs (Rudin et al., 1999). Although, until now both methods are very expensive they have several applications in studies of body composition in live animals.

In growing animals, to obtain better insight in the composition of the live weight gain, the comparative or serial slaughter method is sometimes used. The slaughter method involves the slaughter and individual chemical analysis of a control group at the start of the experiment. Another group is fed a weighed and analyzed diet for a given period and are then slaughtered and analyzed. The difference in their body composition from that of the control animals killed at the start reveals the composition of the gain and the total energy balance (TEB), consequently the difference between the total intake of metabolizable energy (ME) and the total energy balance (TEB) corresponds to the total heat production (HE). Accurate results are only obtained when the number of animals is large enough so that the inevitable variation caused by between-animal variation does not have a great effect. This method is rather expensive and laborious when used for cattle and pigs, but is often used for chickens.

Recently, near infra-red reflectance (NIR) has been introduced as a method to save time, and cost of postmortem body chemical analyses. Such techniques are not mechanistic and fail to account for relevant metabolic processes that convert dietary N and carbon into greenhouse gases (i.e., CO_2, CH_4, and NH_3 or N_2O). To circumvent the limitations of the above techniques and NE system, some researchers (Boisen and Verstegen, 1998b; Whittemore, 1999, de Lange and Birkett, 2004) have proposed to drop the concept of energy, and model the effects on performance according to the utilization of dietary nutrients. Although such a model would integrate feed and animal effects to achieve the best characterization of the effect of a diet on performance, no such model is yet available. Considerable experimentation is required to obtain the data needed to fully and successfully implement such models

2.3 Supplementation of Synthetic Amino Acids to Swine Diets

The use of synthetic amino acids (AA) has become an important part of diet formulation within the swine industry. Synthetic amino acids are relatively purified sources of AA that can be added to diets to meet an AA requirement. Their use not only allows for producers to lower feed cost per unit of gain, but also may help producers in their efforts to manage nutrients more effectively and prevent damage to the environment caused by excess N in manure.

Unfortunately, there are problems that limit the use of synthetic AA. For example, rate and efficiency of growth is usually negatively affected when crude protein (CP) is decreased by more than three percentage units without adequate supplementation of the limiting amino acids to a protein-bound C-SM based diet (Lewis and Southern, 2001). The causes of these negative effects are unknown, but may be a result of one or several factors. When diets are formulated with synthetic AA, the requirement estimates for the next limiting amino acids become crucial, because their concentrations are decreased and are more likely to be nearing a deficiency. Furthermore, there is dearth of information regarding AA requirements in modern genetic strains at varying stages of growth.

2.3.1 Commonly Used Synthetic Amino Acids

Four commercially available synthetic amino acids (lysine, methionine, threonine, and tryptophan) are commonly used in diets for swine. Their use is largely dependent upon price of protein feeds such as canola and soybean meal. Specifically, as the price of protein feeds increases the use of synthetic AA becomes more economical. On the contrary, when soy prices are low the economic returns from using synthetic AA are decreased. In most swine diets the first limiting AA is lysine. Therefore, the use of other synthetic AA may be dependent upon the price of lysine. Additionally, the prices of other synthetic AA directly affect how much synthetic lysine will be used.

Lysine is most commonly sold commercially as L-Lysine monohydrochloride (L-lysine.HCl). There are no known mammals that are able to utilize D-Lysine and therefore only L-Lysine is considered to have bioavailability in swine and poultry (Lewis and Southern, 2001). Feed grade lysine contains a minimum of 98.5% L-Lysine.HCl. This is equivalent to 78.8% actual lysine.

Methionine seems to be equally available for use in swine diets in the D- or L-form (Waldroup et al., 1981; Reifsnyder et al., 1984; Chung and Baker, 1992). Feed grade sources of methionine are available as DL-Met (99% pure) and as Met hydroxy analog (a liquid that contains 88% Met hydroxyl analog). There is considerable controversy over the relative bioactivity of Met hydroxyl analog; however, previous research has indicated that it is equivalent to DL-Met on an equal molar basis (Waldroup et al., 1981; Reifsnyder et al., 1984; Chung and Baker, 1992).

Threonine has four chemical isomers: D- and L-Thr, and D- and L-allothreonine (Lewis and Southern, 2001). There is the assumption that pigs can only utilize L-Threonine, because of an inability for transamination to occur (Lewis and Southern, 2001). Thus, commercially available threonine is in the L-form (98.5% pure).

The bioactivity of tryptophan, in the form D-Trp, varies between species and may vary from 60 to 100% in pigs (Lewis and Southern, 2001). Almost all feed-grade tryptophan is available as L-Trp (98.5% pure).

The use of other synthetic AA in diets for swine will be dependent upon the extent to which CP can be lowered without affecting growth and carcass traits. There is the possibility that in the future, synthetic forms of other essential AA will be produced commercially and priced competitively for inclusion into swine diets.

2.4 Low Protein Amino Acid Supplemented Diets for Swine

Increasing environmental concerns related to N concentrations of swine manure have generated interest in the use of synthetic AA to lower the crude protein content of swine diets. Kerr and Easter (1995) estimated that each one percentage unit reduction in dietary crude protein results in 8% less N excreted in manure. However, there are some inconsistencies in the literature as to the extent crude protein can be lowered without affecting growth performance and carcass traits. Most reports suggest reducing CP by more than 3 to 4 percentage units leads to a reduction in rate and efficiency of growth, even when all known nutrient requirements are met (Tuitoek et al., 1997a, b; Shelton et al., 2001; Gomez et al., 2002a, b). Furthermore, reductions in CP with the use of synthetic amino acids often lead to increased fat deposition (Tuitoek et al., 1997a, b; Knowles et al., 1998; Shelton et al., 2001; Figueroa et al., 2002, Gomez et al., 2002a, b).

Knowles et al. (1998) suggested that pigs fed low CP synthetic AA diets will have a lower energy need for deamination of excess AA and lower pancreatic activity. Therefore, the net energy of the diet increases and this results in fatter carcasses. However, the authors concluded that reduction of net energy in low CP AA supplemented diets was not an effective means of reducing fat in finishing pigs. Other research results suggest that formulating with the net energy system will prevent fatter carcasses (Le Bellego et al., 2001, 2002).

Liu et al. (2001) reported that a 9.55% CP corn diet fortified with free lysine, threonine, tryptophan, methionine, iso-leucine and valine supported growth equal to gilts fed a 15.17% CP corn-soybean meal diet. Liu and co-workers also reported that deletion of synthetic iso-leucine or valine reduced growth performance of gilts. Shelton et al. (2001)

evaluated nine different protein sources for growing-finishing pigs. Diets for their experiment were formulated to meet all NRC (1998) AA requirements and they maintained an equivalent lysine:calorie ratio. They reported feeding a 9.35% CP corn diet with synthetic AA reduced performance compared to the corn-soybeanmeal control diet during the grower and early-finishing periods, but not during the late-finishing period.

According to NRC (1998), the order of limiting AA in a corn diet is as follows: lysine, tryptophan, threonine, iso-leucine, valine, and methionine. Eliminating soybean meal in diets for late-finishing barrows results in close to a 5% reduction in CP. This amount of CP reduction may lead to a deficiency in dispensable N. Kendall et al. (2004) concluded that adding Gln and Gly together, or Glu improved performance of nursery pigs fed low CP diets. However, growth studies conducted by Zimmerman (1975), Taylor et al. (1981), Russell et al. (1987), and Kephart and Sherritt (1990) have all shown no effect on growing pig performance to the addition of dispensable N.

2.5 Potential to Enhance the Nutritional Value of Feedingstuff and Diets

Many feed ingredients including cereals and their by-products, contain considerable amounts of anti-nutritional factors (ANF), which affect the nutritional value of those ingredients when fed to monogastrics. In recent years, concerted efforts to enhance the nutritional value of such feedstuffs have been undertaken to maximize nutrient utilization. The use of exogenous enzymes has been recognized as one of the ways to improve nutrient utilization. Apart from improved nutrient utilization from feedstuffs, the quality of the environment might be improved by reducing the manure output and pollution associated with manure (Classen, 1998).

Although phytase addition has aimed primarily at a reduction in phosphorus excretion, phytase addition to feed may increase protein digestibility and feed efficiency (NRC, 1998). Ketaren et al. (1991) estimated that phytase addition improved growth and protein deposition by 15%, while feed efficiency was improved by 10%. The hydrolysis of phytate by phytase increases the utilization of phytate-bound P (phytate-P) and reduces P excretion, but increasingly research suggests that phytase also improves protein and

energy metabolism (Ravindran et al., 1999a). Possible interactions between phytase and xylanase following their simultaneous inclusion in wheat-based broiler diets have attracted interest in recent years (Ravindran et al., 1999a), but not in pig diets. Despite the increasing likelihood of phytase and xylanase being used simultaneously in practice published reports on their combined application are limited. Also, phytase addition to diets has not been studied under feeding strategies for mitigating GHG emission and no data about its effectiveness on reducing CO_2 and CH_4 is available.

Addition of cellulases and hemicellulases improve animal performance by degrading non-starch polysaccharides (NSP) that may interfere with digestion of other nutrients (Li et al., 1995, 1996). This improvement is primarily in barley/wheat-based diets (Thacker and Baas, 1996), but may also occur in corn-soybean meal diets (NRC, 1998). The beneficial effects of the addition of these enzymes should be a reduction in methane (CH_4) production, which is linearly related to the ingestion of non-starch polysaccharides (NSP) (Jensen, 1996). There are currently no data available in the literature that directly assesses the effect of these enzymes on GHG (especially CH_4) emissions.

2.6 Use of Exogenous Enzymes in Livestock Industry

Several possible mechanisms have been proposed to explain the beneficial effects of the use of exogenous enzymes in livestock industry: (1) removal of anti-nutritional factors (ANF); (2) increasing the digestibility of existing nutrients; (3) increasing the digestibility of NSP; and (4) supplementing host endogenous enzymes (Classen, 1998). Nevertheless, the current primary use for exogenous enzymes in commercial livestock rations is the potential for degradation of NSP or phytic acid to maximize the nutrient utilization from feedstuffs.

2.6.1 Carbohydrase Supplementation of Liveststock Diets

Enzyme preparations have been formulated to facilitate the digestion process of NSP in monogastrics, prior to fermentation in the large intestine. These enzymes are commonly referred to as carbohydrases. The main carbohydrases used in livestock rations to alleviate the detrimental effects associated with NSP are xylanase and β-glucanase. The

effects of carbohydrases on nutrient digestibility and growth performance at different growth stages of non-ruminants, using either xylanase or β-glucanase in poultry and swine diets have been studied in depth. In addition, both carbohydrases combined have been studied (Thacker et al., 1992a; Inborr et al., 1993; Yin et al., 2001a,b; Högberg and Lindberg, 2004; Zijlstra et al., 2004; Thacker and Rossnagel, 2005), making definitions of relationships between specific activities and beneficial effects difficult to interpret. Interestingly, the influence of these supplemental enzymes, mostly in combination with other fibrolytic enzymes, has also been studied in ruminants, despite the fibre degradation ability of microorganisms in the rumen (ZoBell et al., 2000; Bowman et al., 2002; Kung et al., 2002; Colombatto et al., 2003; Yu et al., 2005).

Experiments with swine however, often demonstrated inconsistent responses to xylanase supplementation. Some studies showed an improvement in nutrient digestibility (Rattay et al., 1998; Yin et al., 2000; Barrera et al., 2004) and growth performance (Van Lunen and Schulze, 1996; Barrera et al., 2004), while the other experiments concluded with a lack of response either in nutrient digestibility (Thacker et al., 1991; Bedford et al., 1992; Mavromichalis et al., 2000; Yin et al., 2001b; Diebold et al., 2005) or performance (Bedford et al., 1992; Inborr et al., 1993; Mavromichalis et al., 2000). Due to the inconsistent responses, future research should employ more precise and sensitive methods than growth rate or crude nutrient digestibility.

2.6.2 Carbohydrase Supplementation of Swine Diets

Numerous attempts have been made to maximize nutrient digestibility and hence improve the performance of pigs fed diets based on cereal grains supplemented with exogenous carbohydrases. Nevertheless, most of these studies have not been successful in demonstrating improvements in performance of a magnitude similar to those observed in poultry for either starter pigs (Inborr and Ogle, 1988; Officer, 1995; Thacker et al., 1992b; Inborr et al., 1993; Baidoo et al., 1998; Jensen et al., 1998; Li et al., 1999; Mavromichalis et al., 2000; Högberg and Lindberg, 2004) or grower-finisher pigs (Newman et al., 1980; Thacker et al., 1989; Thacker et al., 1992a, 1992b; Baas and

Thacker, 1996; Thacker and Campbell, 1999; Mavromichalis et al., 2000; Thacker and Rossnagel, 2005).

Recently, the suppressive effect of NSP on voluntary feed intake (VFI) received attention. A positive quadratic dose-response correlation existed between average daily feed intake (ADFI) and average daily gain (ADG) with carbohydrase supplementation of starter pig diets, indicating that carbohydrase will improve VFI, but suggesting that an excess breakdown of the respective NSP in the gastro-intestinal tract may thereafter directly or indirectly inhibit voluntary feed intake (Zijlstra et al., 2004). On the other hand it could mean that net energy was initially limiting in the diet fed to these pigs, other wise an increase in growth would not have occurred.

Supplemental carbohydrases have been tested for their ability to improve nutrient digestibility in swine. A number of previous studies reported no improvement in nutrient digestibility either in starter (Bedford et al., 1992; Thacker et al., 1992b; Li et al., 1996b; Mavromichalis et al., 2000; Högberg and Lindberg, 2004; Zijlstra et al., 2004; Diebold et al., 2005) or grower-finisher pigs (Graham et al., 1986, 1989; Thacker et al., 1989; Thacker et al., 1992a, 1992b; Li et al., 1996a; Mavromichalis et al., 2000). Under normal feeding conditions and with good quality cereal ingredients, beneficial effects of carbohydrases on nutrient digestibility and growth performance have been consistently demonstrated (Zijlstra et al., 2004). However, some beneficial effects of carbohydrases on nutrient digestibility and growth performance have been obtained from cereals grown under harsh climatic and soil conditions, and of poor quality (Thacker et al., 1992b; Li et al., 1996a; Mavromichalis et al., 2000). Due to the inconsistent responses, future research should employ more precise and sensitive techniques and measure variables other than growth rate and crude nutrient digestibility.

2.7 Phytate and Action of Phytase

Phytate (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) is an anionic acid with anti-nutritional properties, which is present in many feed ingredients used for livestock. The main anti-nutritional effect of phytate is to make P essentially unavailable

for digestion and absorption by non-ruminants (Nelson, 1967). Phytate also has negative effects on other nutrients. Phytate may influence starch digestibility through interaction with amylase, proteins associated with starch, Ca (which catalyzes amylase activity), or with starch itself (Sharma et al., 1978; Deshpande and Cheryan, 1984; Thompson and Yoon, 1984; Thompson et al., 1987; Johnston et al., 2004). Phytate also has been shown to form complexes with both dietary and digestive proteins (Okubo et al., 1976; Hartman, 1979; Satterlee and Abdul-Kadir, 1983) and to inhibit trypsinogen (Caldwell, 1992); thus affecting the protein nutritional quality of feed ingredients. Phytate has been shown to form complexes with trace minerals and decrease their availability (Maddaiah et al., 1964; Vohra, 1965; Davies and Nightengale, 1975; Erdman, 1979; Ravindran et al., 1995), but the reported order of affinity that trace minerals bind to phytate varies. Maddaiah et al. (1964) reported that phytic acid affects Zn > Cu > Co > Mn, but Vohra (1965) indicated that phytate forms complexes with Cu > Zn > Ni > Co > Mn > Fe.

Phytase is an enzyme that breaks down phytate (Gibson and Ullah, 1990) allowing the release of nutrients bound to phytate and increasing their availability for digestion and utilization. There is ample research on the effect of phytase on P and Ca availability (Johnston, 2000; Kornegay and Verstegen, 2001), but there is much less research regarding protein and energy efficiency.

2.7.1 Effect of Phytase on Energy Metabolism in Swine

Because of the above-mentioned effect of phytate on protein, starch, and minerals, the addition of phytase to the diet may have positive effects on energy availability for swine and poultry.

Research has indicated that phytase may have positive effects on energy availability in diets for chicks (Rojas and Scott, 1969; Miles and Nelson, 1974; Namkung and Leeson, 1999; Ravindran et al., 1999b, 2000, 2001; Camden et al., 2001; Selle et al., 2003b; Shirley and Edwards, 2003). However, other reports have indicated that phytase has no effect on energy availability (Biehl and Baker, 1997; Ledoux et al., 2001; Murai et al., 2002). Selle et al. (2003c) reported that adding phytase to wheat-sorghum-SBM based

diets increased chick apparent ME in one experiment, but had no effect in two others. Johnston and Southern (2000a) indicated that phytase (600 phytase units/kg) provided 45.7 kcal of ME/kg in C-SBM diets for chicks. However, the ME matrix values for Natuphos® indicates it provided 31 and 24 kcal/kg for starter and growing-finishing broilers respectively (Johnston and Southern, 2000a).

There is little research on the effects of phytase on energy availability in pigs. Murry et al. (1997) reported no effect on digestible energy when 700 phytase units/kg of diet were added to pearl-millet-SBM-based diets, but an increase in digestible energy when 1,000 phytase units/kg of diet were added. Johnston et al. (2004) reported that phytase addition increased apparent ileal gross energy digestibility in pigs with no effect on total-tract energy digestibility. O'Doherty et al. (1999) indicated that adding phytase to finishing pig diets increased energy digestibility. However, Murry (1994), O'Quinn et al. (1997), Gerbert et al. (1999b), Walz and Pallauf (2002), and Sauer et al. (2003) reported that adding phytase had no effect on energy digestibility in pigs. The ME matrix values for Natuphos® indicates it provides 23 and 26 kcal/kg for starter and growing-finishing pigs, respectively.

Based on this review of the literature, phytase has had variable effects on energy digestibility, but some reports have suggested possible modes of action other than its effect on minerals and protein digestibility. Phytase may increase energy availability by increasing the availability of carbohydrates. Johnston et al. (2004) reported that adding phytase to diets for the growing pig increased starch digestibility and plasma levels of glucose and insulin 30 min after a meal. However, Walz and Pallauf (2002) indicated that phytase addition to chick or swine diets respectively had no effect on plasma glucose concentration

Some reports indicate that phytase may increase energy digestibility by increasing fat digestion. In chicks, fat digestion has been shown to be increased with phytase addition (Um et al., 2000; Lim et al., 2001). Phytase addition to a pea-based diet for growing pigs increased crude fat and carbohydrate digestibility (Helander et al., 1996), but Brady et al.

(2003) and Johnston et al. (2004) indicated that phytase addition to growing-finishing pig diets had no effect on fat digestibility. If the positive effects of phytase on energy are correct, the response may be due to additive effects on fat, starch, protein, and minerals. Therefore, the application of calorimetry which is more sensitive and precise than the measures previously applied, may help resolve the issue of whether phytase addition can increase energy availability for swine.

2.7.2 Effect of Phytase on Protein and Amino Acid Metabolism in Swine

Phytase has the potential to improve the environment by causing a decreased excretion of P and N, which in excess can be major pollutants from animal waste. Because of this, it is important to determine the effect of phytase on protein digestibility, which will allow nutritionists to formulate diets closer to the animal's requirement, thus reducing N in the excreta. Phytase may increase the availability of protein in swine and poultry diets, not only by breaking the phytate-protein complex, but also by reducing the amount of inorganic P added to the diet. Nasi (1990) reported that adding inorganic P to the diet for growing pigs reduced N digestibility.

Research supporting the effect of phytase addition on N (CP) availability is reported in the literature. Phytase also has been shown to have variable effects on the protein efficiency ratio (PER), which can be used as a measure of protein quality of common feed ingredients. Boling-Frankenbach et al. (2001) indicated no effect of phytase supplementation on the PER of soybean meal (SBM), corn gluten meal, canola meal, casein, cottonseed meal, peanut meal, wheat bran, wheat middlings, rice bran, defatted rice bran, or meat and bone meal in chicks. In that study, adding Ca and P did not affect the PER relative to those fed a positive control diet. Also, Selle et al. (2003b) reported that adding phytase to wheat-sorghum based diets did not affect PER in 7 to 25 d old chicks, but increased PER in 0 to 42 d old chicks. Peter and Baker (2001) indicated that phytase addition to low CP-SBM-based diets had no effect on PER of chicks.

As mentioned earlier, phytate has the potential to form complexes with amino acids (AA), thus making them unavailable. Because of this, research has been conducted on the

effects of phytase on AA availability in swine and poultry. Rutherford et al. (2002) and Ravindran et al. (1999a) reported that phytase addition increased the availability of most AA in corn, SBM, wheat, rice bran, rapeseed, sorghum, cottonseed meal, canola meal and sunflower meal for chicks. In the study by Rutherford et al. (2002) the availability of all AA were at least numerically increased with phytase addition. Johnston and Southern (2000b) reported no effect of phytase addition on overall AA digestibility in chicks, but the addition of phytase to low Ca and P diets did increase the availability of Lys, Ile, and Leu. Ravindran et al. (1999b) indicated that Lys, Thr, and Ser digestibilities were increased in chicks fed diets with added phytase. Namkung and Leeson (1999) indicated that phytase addition to chick diets increased total AA and non-essential AA ileal digestibility, and specifically increased the availability of Val and Ile. Also, Ravindran et al. (2000) reported that adding phytase to low, medium or high phytic acid diets for chicks increased AA digestibility, and as expected, phytase had more of an effect in diets with high phytic acid. Sebastian et al. (1997) reported that phytase had a greater effect on AA digestibility in female chicks (increased digestibility of all AA except for Lys, Met, Phe, and Pro) relative to male chicks. Johnston et al. (2004) reported that adding phytase to diets for growing pigs increased total AA digestibility relative to those fed a control diet. Mroz et al. (1994) reported that adding phytase to pig diets increased apparent total tract digestibility of AA (all AA were numerically increased except for Cys and Pro). Murry et al. (1997) reported that adding phytase to pig diets with two different levels of P increased apparent digestibility of Lys, Leu, Ile, and Val. In contrast, some reports in swine indicate no effect of phytase addition on AA digestibility. Valaja et al. (1998) indicated that adding phytase to a semi-purified diet for finishing pigs had no effect on apparent total tract or ileal AA digestibility. Also, Omegbenigun et al. (2003) reported no effect on phytase addition to low P diets on essential AA digestibility (except for His) in early-weaned pigs.

The above-mentioned research has been conducted with phytase addition to diets that were adequate in CP, but because of the increased interest in reducing the amount of P and N in animal waste, phytase addition in low CP diets could have a major impact on the environment. Phytase has been added to swine and poultry diets with low CP and/or AA

levels with varying effects on AA availability. Some studies indicate an increase in AA digestibility (Biehl and Baker, 1996; Biehl and Baker, 1997; Kornegay et al., 1998; Radcliffe et al., 1999; Zhang et al., 1999; Ravindran et al., 2000; Ravindran et al., 2001; Camden et al., 2001; Ledoux et al., 2001; Selle et al., 2003a; Selle et al., 2003c) while others have reported no effect (Zhang et al., 1999; Peter et al., 2000; Brumm, 2001a; Grandhi, 2001; Traylor et al., 2001; Walz and Pallauf, 2002).

To properly formulate AA in diets with phytase, we must know the correct amount of Lys that is released by phytase. Research has shown that phytase provides an additional 0.023% (Johnston and Southern, 1999) or 0.064% (Ravindran et al., 2001) Lys for chicks, and 0.03% Lys for 48-kg pigs (Radcliffe et al., 1999). Also, phytase has been shown to provide 0.76% CP for finishing pigs (Zhang and Kornegay, 1999) and 0.52% CP for 48-kg pigs (Radcliffe et al., 1999). Therefore, for Natuphos®, the matrix value used for CP and Lys are underestimated. For chicks, the CP and Lys matrix values are 0.21 and 0.014% and 0.24 and 0.016% for starter and grower-finisher broilers, respectively. For swine, the CP and Lys matrix values are 0.28 and 0.013% and 0.31 and 0.014% for starter and growing-finishing pigs, respectively.

Future research would have to employ other preparations of phytase with well estimated matrix value. Also, there is the need to employ the application of calorimetry, which is more sensitive and precise than measures currently employed, to help resolve the issues of phytase and energy availability.

2.8 Non-starch Polysaccharides and Action of Xylanase

The cell walls of cereal grains are primarily comprised of complex carbohydrates referred to as NSP (Choct, 1997) , and consist predominantly of β-glucans in barley and oats, and arabinoxylans in wheat, rye and triticale (Englyst et al., 1989; Bach Knudsen, 1997; Zijlstra et al., 1999). The β-glucans consist of linear chains of β-glycosyl residues linked by β (1-3) or (1-4) bonds with predominant (1-4) linkages (MacGregor and Fincher, 1993). Arabinoxylans, on the other hand, are β (1-4) linked polymers of the pentoses arabinose and xylose, and are hence referred to as pentosans (Fincher aand Stone, 1986).

The predominant NSP in cell walls varies among cereal grains. Furthermore, the content of NSP varies widely among cereals, with barley and hulless barley containing more NSP than wheat and corn (Fincher and Stone, 1986). Finally, the NSP content varies among samples of barley (Fairbairn et al., 1999), wheat (Zijlstra et al., 1999) and hulless barley (Andersson et al., 1999). Not only grain type and cultivar but also the rate of fertilization and growing conditions may affect the NSP content within a crop year (Oscarsson et al., 1998).

The NSP fraction of cereal grains has drawn a considerable attention in poultry and swine ration formulation, because the digestive system of monogastrics lacks the appropriate endogenous enzymes required to hydrolyse NSP (Li et al., 1996a,b; NRC, 1998). In poultry, NSP-associated digesta viscosity is mainly responsible for the reduced nutrient utilization and growth performance (Choct and Annison, 1992; Almirall et al., 1995). Nevertheless, viscosity-altering properties of NSP are not as likely to be an issue of the same magnitude in swine nutrition (Bedford et al., 1992; Johansen et al., 1997; Pluske et al., 1999; Mivromichalis et al., 2000; Medel et al., 2002; Högberg and Lindberg, 2004; Zijlstra et al., 2004). Rather, as a physical barrier, interferences of NSP with the action of digestive enzymes and digestion process are more important (van Barneveld et al., 1995; Grieshop et al., 2001). The negative correlation between the energy digestibility and the dietary content of NSP is well-documented (Fairbairn et al., 1999; Zijlstra et al., 1999; de Lange et al., 2000; Yin et al., 2002. In swine, apparent total-tract NSP digestibility is higher than apparent ileal digestibility, due to microbial degradation in the hindgut (Li et al., 1996a, b). From a nutrient utilization point of view, however, energy sources absorbed after fermentation in the large intestine have less nutritional value for pigs than energy sources absorbed in the small intestine after digestion with endogenous digestive enzymes (Noblet et al., 1994; Fuller and Reeds, 1998; Noblet, 1999). Moreover, the net energy value of carbohydrates fermented and absorbed as volatile fatty acids in the large intestine of pig is 30% lower than that from the small intestine (Dierick et al., 1989).

Therefore, there is need for research applying calorimetry, which is more sensitive and precise than the measures currently applied. This may help resolve the issue of xylanase and energy availability.

2.8.1 Xylanase Supplementation of Swine Diets

A considerable amount of recent research with carbohydrases has been conducted using supplementary xylanase, which is generally used in wheat-based diets. Nevertheless, studies to evaluate the effect of supplementary xylanase on nutrient digestibility and performance in wheat-based diets for swine are limited and controversial.

While a number of studies reported beneficial effects of xylanase on pig growth performance (Dierick, 1989; Van Lunen and Schulze, 1996; Barrera et al., 2004; Zijlstra et al., 2004), other studies reported no significant effects (Thacker et al., 1991, 1992a; Bedford et al., 1992; Inborr et al., 1993; Thacker and Baas, 1996; Högberg and Lindberg, 2004; Thacker and Rossnagel, 2005). Inconsistent results have also been reported among weight categories in swine. For example, xylanase supplementation did not affect performance in nursery pigs and in one of two experiments with finisher pigs fed wheat-based diets; however, xylanase improved performance in the second experiment with finisher pigs (Mavromichalis et al., 2000). Similarly, supplemental xylanase affected nutrient digestibility in pigs inconsistently. Sometimes xylanase improved digestibility (Graham et al., 1988; Baidoo et al., 1998; Rattay et al., 1998; Yin et al., 2000b; Barrera et al., 2004; Thacker and Rossnagel, 2005), whereas sometimes nutrient digestibility of pigs did not improve with supplementary xylanase (Thacker et al., 1991, 1992a; Bedford et al., 1992; Mavromichalis et al., 2000; Yin et al., 2000; Yin et al., 2001; Högberg and Lindberg, 2004, Zijlstra et al., 2004; Diebold et al., 2005).

One unit (U) of xylanase activity is defined as the amount of enzyme required to release 1 mol of reducing sugars (expressed as xylose) in 1 min (Tervila-Wila et al., 1996). Xylanase supplementation at rates of 5,500, 11,000 and 16,500 U kg^{-1} improved nutrient digestibility and some performance variables linearly and quadratically in grower pigs fed wheat-based diets, with the best response with the intermediate inclusion level of

xylanase (11,000 U kg^{-1}; Barrera et al., 2004). In another experiment conducted using finishing pigs fed wheat-based diets in mash and pellet forms supplemented with two different forms of xylanase, powder or liquid with 4,000 or 8,000 U g^{-1} xylanase activity, respectively, xylanase improved growth performance and nutrient digestibility in pigs fed mash diets but not in pigs fed pellet diets (Park et al., 2003). Inconsistency in growth performance and nutrient digestibility in pigs fed diets with supplementary xylanase has been attributed to the factors such as growth stage of pigs, level of xylanase and the nature of the diet.

2.8.1.1 Effect of Xylanase on Energy Digestibility

Xylanase supplementation has improved energy digestibility in some studies involving pigs, whereas an equal number of the studies lacked a beneficial of response. The inconsistency may be attributed to variations among the major ingredients in their NSP content, the level of supplemental xylanase, the age of experimental pigs and the lack of sensitivity of methods used.

Supplemental xylanase in diets based on wheat and its by-products improved ileal and total-tract apparent energy digestibility by 3 and 1%, respectively, in grower pigs (Yin et al., 2000b). In weanling pigs, xylanase supplementation in combination with β-glucanase in diets based on hull-less barley improved both apparent ileal and total-tract digestibility of energy by 11 and 6%, respectively (Baidoo et al., 1998) and of diets based on barley and pollard, improved the apparent ileal digestibility of energy by 4%, without affecting total-tract digestibility (Graham et al., 1988). In addition, the same enzyme combination improved the total-tract digestibility of energy in grower-finisher pigs fed diets containing normal or high fat oats (Thacker and Rossnagel, 2005).

In contrast, xylanase supplementation in meal and pellets of rye-based diets did not improve total-tract energy digestibility in weanling pigs (Thacker et al., 1991). Similarly, supplementing xylanase to diets based on hulless barley or wheat did not improve either apparent ileal or total-tract energy digestibility in young or weaner pigs, respectively (Yin et al., 2001b; Diebold et al., 2005). Supplementing diets based on barley, wheat, oats,

triticale and wheat bran with xylanase together with β-glucanase did not affect total tract digestibility of energy in weaned piglets (Högberg and Lindberg, 2004). The same enzyme combination at different inclusion rates in diet based on wheat and canola meal did not affect apparent digestibility of energy in any of the four segments of the small intestine examined in weaned pigs (Zijlstra et al., 2004). Moreover, xylanase in combination with β-glucanase did not influence total-tract energy digestibility of growing and finishing pigs fed diets based on barley or rye (Thacker et al., 1992a). Interestingly, xylanase together with β-glucanase did not affect the total-tract digestibility in grower pigs fed diets containing barley, wheat, SBM and low-mucilage canola meal, but improved digestibility coefficients in pigs fed the same diet, replacing low-mucilage canola meal with Candle canola meal (Bell and Keith, 1991). Combined, the data suggest that effects of xylanase on energy digestibility are dose dependent. However, it may also depend on the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting energy digestibility in the specific diet.

Total tract digestibility should be considered a poor method because it lacks sensitivity and precision as a primary outcome measure, since endogenous and microbial losses in the hindgut are not accounted for. Therefore, the application of calorimetry, which is more sensitive and precise than the measures currently applied, in future research may help resolve the issue of xylanase and energy availability. Additionally, future research may use newer commercial preparations of xylanase.

2.8.1.2 Effect of Xylanase on Protein and Amino Acid Digestibility

Beneficial effects of xylanase supplementation on protein and AA digestibility in swine have been observed. With the increase in the rate of xylanase supplementation, apparent ileal digestibility of CP and AA improved linearly and quadratically in grower pigs fed wheat-based diets, achieving the highest digestibility values in diets supplemented with 11,000 U g^{-1} enzyme activity, improving CP digestibility by 7% (Barrera et al., 2004). Supplementation of a diet containing barley, wheat, wheat bran and SBM with xylanase improved apparent ileal digestibility of some of the indispensable AA and of CP by 2% and total-tract digestibility of CP by 1% in grower pigs (Yin et al., 2000b).

Supplementary xylanase in combination with β-glucanase in two diets, each containing one of two varieties of hull-less barley, improved both apparent ileal and total tract digestibility of CP by 7% and 9%, respectively, and apparent ileal digestibility of both diets (Baidoo et al., 1998). In addition, supplementing xylanase together with β-glucanase, improved the apparent ileal digestibility of CP by 7%, without affecting total tract digestibility, in weanling pigs fed diets based on barley and pollard (Graham et al., 1988). The same enzyme combination improved the total tract digestibility of CP in grower-finisher pigs fed diets containing normal or high fat oats (Thacker and Rossnagel, 2005).

In contrast, supplementing xylanase in rye-based diets did not show any improvement in CP (N) digestibility in weanling pigs (Bedford et al., 1992). Likewise, xylanase supplementation to diets based on wheat did not affect apparent ileal or total digestibility of CP or ileal digestibility of AA in weaner pigs (Diebold et al., 2005) and total tract digestibility of CP (N) in nursery and finishing pigs (Mavromichalis et al., 2000).

Supplementary xylanase in combination with β-glucanase had no influence on CP digestibility at any site of digestive tract of weaned piglets fed diets based on main cereals and wheat bran (Högberg and Lindberg, 2004). The same enzyme combination did not influence the total tract CP digestibility of growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al., 1992a), or of growing pigs fed diets containing barley, wheat and SBM with either low-mucilage or candle canola meal (Bell and Keith, 1991). In addition, supplementing hull-less barley-based diets with xylanase did not affect apparent ileal or total tract digestibility of CP in young pigs, but improved apparent ileal digestibility of most of the indispensable and dispensable AA (Yin et al., 2001).

As was the case for energy digestibility, plausible factors contributing to the inconsistency in AA and CP digestibility among studies may be the variations in substrate level of ingredients used, the level of supplementary xylanase added and the variations in genetic potential and age of experimental pigs. Combined, the data suggest

that effects of xylanase on protein and AA digestibility may depend on the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting protein and AA energy digestibility in the specific diet.

Therefore, to elucidate the plausible factors contributing to the inconsistencies in the literature future research may need the application of calorimetry, which is more sensitive and precise than measures currently employed, to help resolve the issues of xylanase and energy availability.

2.8.1.3 Effect of Xylanase on Dry Matter Digestibility

Effects of xylanase supplementation on DM digestibility have been mixed, with only some studies showing a favourable response. Addition of xylanase to diets based on wheat or its by-products improved apparent ileal and total-tract DM digestibility by 4% and 1%, respectively, in grower pigs (Yin et al., 2000b). In weanling pigs, xylanase supplementation in combination with β-glucanase of diets containing hull-less barley improved both apparent ileal and total tract digestibility of DM by 15% and 6%, respectively (Baidooo et al., 1998) and of diets based on barley and pollard improved the apparent ileal digestibility of DM by 5% without affecting total tract digestibility (Graham et al., 1988). In addition, the same enzyme combination improved the total tract digestibility of DM in grower-finisher pigs fed diets containing normal or high fat oats (Thacker and Rossnagel, 2005).

Xylanase supplementation did not affect DM digestibility in weanling pigs fed meal or pellet for rye-based diets (Thacker et al., 1991). Furthermore, addition of xylanase to wheat-based diets did not improve either ileal or total tract dry (organic) matter digestibility in weaner pigs (Diebold et al., 2005) or total tract digestibility of DM in nursery and finishing pigs (Mavromichalis et al., 2000). Xylanase in combination with β-glucanase, in diets containing a variety of cereals, lowered dry (organic) matter digestibility by 50% in the stomach of weaned piglets, without affecting the digestibility of organic matter at the other sites of the digestive tract (Högberg and Lindberg, 2004). The same enzyme combination did not influence the apparent digestibility of DM in any

of the four segments examined of the small intestine of weaned pigs fed wheat- and canola meal-based diets (Zijlstra et al., 2004). Moreover, supplementary xylanase together with β-glucanase did not influence total tract digestibility of DM in growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al., 1992a). Finally, supplementing hull-less barley diets with xylanase did not affect apparent ileal or total tract digestibility of DM in young pigs (Yin et al., 2001).

Therefore, future research may need the application of calorimetry, which is more sensitive and precise than measures currently employed, to help resolve the issues of xylanase and energy availability, in combination with balance studies.

2.8.1.4 Effect of Xylanase on Animal Performance

The consequences of xylanase supplementation on pig performance have been variable. Supplemental xylanase in corn- and wheat-based diets improved ADG by 9.2%, ADFI by 4%, and feed efficiency by 5.3% in grower pigs (Van Lunen and Schulze, 1996). With different rates of xylanase supplementation, ADG and feed efficiency were improved linearly and quadratically in grower pigs fed wheat-based diets, achieving the highest responses with diets supplemented with 11,000 U g^{-1} xylanase (Barrera et al., 2004). In another experiment, different rates of xylanase supplementation in combination with β-glucanase increased ADFI, ADG and body weight quadratically in weaned pigs fed diets based on wheat and canola meal, achieving the best performance with the diet supplemented with 2 g kg^{-1} carbohydrase, resulting in 16, 13, and 7% higher ADFI, ADG and body weight, respectively, compared to the control diet (Zijlstra et al., 2004). However, enzyme supplementation reduced feed efficiency linearly. In addition, the same combination of supplementary enzymes improved ADG by 5%, without affecting ADFI or feed efficiency, of growing pigs fed diets containing barley, wheat, SBM and canola meal (Bell and Keith, 1991).

In contrast, supplementing xylanase to rye-based diets in meal and pellet forms did not affect the ADG or ADFI in weanling pigs, but enzyme addition to the diet in meal form improved feed efficiency by 10% (Thacker et al., 1991). Furthermore, xylanase

supplementation did not improve performance of weanling pigs fed rye-based diets (Bedford et al., 1992). Similarly, xylanase supplementation did not improve performance of weaning pigs fed wheat- or barley-SBM diets (Inborr et al., 1993). Xylanase supplementation did not improve performance in nursery or finisher pigs fed wheat-based diets (Mavromichalis et al., 2000). Supplementary xylanase in combination with β-glucanase did not influence the performance of growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al., 1992a), or of grower-finisher pigs fed diets containing normal or high fat oats (Thacker and Rossnagel, 2005). Moreover, the same enzyme combination did not affect the feed intake or ADG of weaned piglets fed diets composed of main cereal grains and wheat bran (Högberg and Lindberg, 2004).

Therefore, to elucidate the plausible factors contributing to the inconsistencies in the literature, future research may need the application of calorimetry, which is more sensitive and precise than measures currently employed, to help resolve the issues of effect of xylanase on energy digestibility or availability.

2.9 Potential Applications and Implications of Research on Energy and Protein Metabolism

From the literature, reviewed, a reduction of CP of more than three percentage units protein-bound diets for swine often leads to reductions in performance and/or negative effects on carcass composition. There is no research conducted that shows any positive impact of reduction of CP of more than five percentage in diets.

Phytase generally has positive effects on energy availability in swine and poultry, although more research has been conducted with poultry. However, there is little research as to whether this increase in energy results in an increase in protein deposition, assuming energy and/or AA is not limiting in the control diet. Research has sometimes shown that phytase also has positive effects on CP and AA digestibility, which may have a major impact on the environment. Increasing the availability of CP and AA in diets for animals will lead to a decreased amount of N in the waste. This effect, along with phytase reducing the amount of P in the waste, can result in reduced levels of nutrients applied to

the land when used for fertilizer. The effect of phytase on AA availability is a much debated topic in swine and poultry nutrition. Phytase seems to have small positive effects on AA. However, this small increase in availability often leads to no statistical effect on individual AA availability, but increases when the AA availability is expressed as total AA, essential AA, or non-essential AA. There is still a need to determine to what degree amino acids are affected by dietary phytase addition. The positive effects phytase has on energy, protein and trace minerals do not appear to lead to any major effects on carcass traits or pork quality. However, most of the results on carcass traits and pork quality are variable and may be due to the reduction in inorganic P supplementation in the control diet rather than the addition of phytase.

The addition of carbohydrases to pig feed has been studied predominantly under the aspect of improving the digestibility of nutrients, namely non-starch polysaccharides. The bulk of experiments have been conducted with growing pigs. To the best of our knowledge, effects of carbohydrase addition on energy metabolism and greenhouse gas emissions have not been studied. In theory, the net energy derived from fermentation and subsequent absorption of volatile fatty acids is 63% or less of the energy derived from enzymatic digestion of polysaccharides (Bakker, 1996). During the fermentation of polysaccharides, up to 18% of the digestible energy may be lost as methane. Therefore, shifting the digestion of non-starch polysaccharides from the hindgut to the small intestine by carbohydrase addition may have two positive effects: improve the energy utilization by pigs and reduce their methane production.

With the introduction of exogenous enzymes to alleviate the detrimental effects associated with NSP in feed ingredients, xylanase may become widely used in commercial livestock rations to maximize the nutrient utilization from feedstuffs. Based on concentrated NSP components in wheat and its by-products, use of supplementary xylanase would be worthwhile; however, the results of the studies conducted to evaluate xylanase supplementation of wheat-based diets on nutrient digestibility and growth performance of pigs are controversial and inconsistent. Plausible factors contributing to the inconsistency in nutrient digestibility among studies might be the variations in

substrate level of ingredients used, level of supplementary xylanase added and the variation in genetic potential and age of experimental animals.

When changing from DE (or ME) to NE systems and using LP diets, attention should be paid to the reduction of amino acid levels and the subsequent risk of deficiency in available amino acid supplies to the pigs. Consequently, it is highly desirable to adopt the *most accurate protein evaluation system (available or digestible amino acids) when a NE* system is used and/or CP level is reduced, in order to adapt feed composition more precisely to requirements and growth potential of the pig. Another consequence is that the utilization of LP diets may result in fatter carcasses due to the higher NE value. Reduction of CP level with sufficient supplies of essential amino acids must then be combined with strategies for obtaining optimum protein and energy intakes, especially with animals that may deposit larger amounts of fats (castrates).

Prediction of net energy value of pig feeds depends directly on their digestible (or metabolizable) energy or digestible nutrients contents. These latter quantities depend firstly on dietary characteristics of the feed, but they are also affected by (bio)-technological treatments, animal factors and interactions between these factors and feed composition. Improvements in the prediction of energy value of pig feeds will therefore come mainly from a better knowledge of factors of variation of energy and nutrient digestibility. In the above paragraphs, we have particularly noted the differences between adult sows and growing pigs which do not appear to be widely recognized by swine producers.

One major objective in the reduction of CP level in diets for growing-finishing pigs or sows is to reduce the nitrogen output from pig production. This can be achieved with good knowledge of amino acids and energy requirements of animals and accurate estimation of the nutritional value of feeds (available amino acids, net energy). Another objective in reducing the CP level (and/or increasing the fat content) might be to decrease the amount of heat which is dissipated by the animals. A potentially high heat production can represent a limiting factor in pigs kept under hot climatic conditions or with a limited

heat tolerance; they will react by a lower VFI and subsequent reduced performance. The use of low CP and/or high fat diets is an attractive solution to reduce heat stress (Lopez et al., 1994). Such strategies deserve further experimental studies and confirmation.

For the Canadian pig industry, the NE system has important implications. The introduction of the NE system would lead to lower feed cost, which would enhance Canada's competitiveness on the international market by regaining the feed cost advantage. The reduction in feed cost would be greater for the more complex diets typically fed in the Prairie region than for simple diets based on corn and soybean meal, typical on American farms. Furthermore, the different valuation of feedstuffs under the NE system would make it more economical to use some domestic rather than imported feedstuffs. For example, the cost of energy in peas decreases from about 40% to less than 30% of the cost of energy in soybean meal when switching from DE to NE. This may apply in a similar manner to other domestic crops with limited content of high-quality protein.

The NE contents of feedstuffs are lower than the DE or ME contents. This means that the energy requirements of pigs must be adjusted to express requirements and feed characteristics in the same system. A simple means of adjustment is to multiply the pig requirements for DE or ME by 0.71 or 0.74, respectively (Noblet et al., 1994). A more accurate and better way is to calculate the pigs' energy requirements factorially as the requirement for maintenance plus the energy content in body protein (23.7 kJ/g, ARC, 1981) and fat (39.6 kJ/g, ARC, 1981). The energy content of the diet would therefore be calculated as daily energy requirement divided by daily feed intake. Full application of NE therefore requires accurate measurement of maintenance energy requirement of Canadian pigs and of on-farm feed intake. There are no published maintenance energy requirements in modern Canadian pigs; calorimetry is required to determine these values. This approach gives a better control over energy content of diets and can be used to control carcass fatness in slaughter pigs.

The benefits are greater with NE than with DE or ME, because the latter do not allow for differences in energetic efficiencies between nutrients. The NE system would: (a) provide a more realistic estimate of dietary energy than either the DE or ME system; (b) allow a better estimate of the effects of diet on performance because diet formulation will be affected by the lower energy value for protein and fibre, and the greater value for starch and fat in the NE system affect diet formulation; (c) allow greater use of cheap feedstuffs and limited use of expensive protein-rich feedstuffs leading to reduced feed costs and lower protein contents, thus reducing N excretion and the environmental impact of pig production; (d) justify the different valuation of feeds which will favor domestic crops, like peas over imported ones, like soybean meal. Thus, all together, a switch to the NE system will improve the economics of both Canadian pig and crop production. Therefore, a shift to NE will help the Prairies regain traditional cost of feeding advantage relative to their major competitors.

2.10. Summary

Low protein amino acid-supplemented diets are commonly used with growing-finishing pigs as a means to reduce N excretion. Lowering dietary protein by 2 pu has no detrimental effect on the performance of growing-finishing pigs and will reduce the N excretion, relative to a conventional diet by 20% (Lenis and Jongbloed, 1999). LP amino acid-supplemented diets have not been intensively examined for reproducing sows, although these animals comprise a considerable portion of the swine population. If LP amino acid-supplemented diets are to be used for sows, a clear understanding of their effects on reproductive performance parameters must be available. The effect of nutritionally adequate LP, amino acid-supplemented diets on milk yield and composition is not well known. Mennega (1986) and Renaudeau and Noblet (2001) reported that neither milk production nor composition was affected by feeding LP amino acid-supplemented diets. This observation needs to be re-confirmed to achieve commercial acceptance of LP diets supplying amino acids as recommended.

LP diets are capable of supporting similar performance in gestating and lactating sows, provided there is adequate supplementation of limiting amino acids. However, the body

research examining LP, amino acid-supplemented diets in sows is extremely limited, and further research must be conducted before such diets will be successful enough to be widely accepted. For example, current gestation research is lacking, and as of yet, no studies appear to have examined feeding LP, amino acid-supplemented diets during both gestation and lactation, and during consecutive parities.

Lack of detailed empirical knowledge of the amino acid requirements, metabolism, and digestion during gestation and lactation is a main limitation to extensive utilization of LP diets. Special attention must be given to amino acid and energy requirements when formulating LP diets, because minor deficiencies of either can impair performance. As we improve our knowledge of how amino acid requirements are influenced by physiological state and metabolism of the sow, by environment and by diet composition, commercial use of LP diets may become more accepted.

Increasing pressure from the public to examine the environmental consequences of intensive swine production will, likely, fuel further studies in this area. Even small decreases in dietary CP content coupled with formulation for a better balance of amino acids will result in considerable decreases in nitrogen excretion (Han and Lee, 2000). Similarly, as the price of crystalline amino acids decreases, greater quantities can be incorporated into practical swine diets without increasing feed costs.

2.11 Hypotheses and Objectives

Based on the gathered information and lack thereof, the following hypotheses were developed: (1) enhancing efficiency of carbon and nitrogen will reduce greenhouse gas emissions from sows and growing-finishing pigs; (2) enhancing carbon and nitrogen retention via phytase and xylanase will increase efficiency of performance in growing-finishing pigs. To address these hypotheses a series of experiments were therefore designed and conducted to evaluate the most promising approaches to: (1) evaluate the effect of diet ingredients and protein level on CO_2 and CH_4 emission and nitrogen and carbon excretion by sows and growing-finishing pigs; (2) assess the effect of high and low protein diets based on corn/soybean (CS) or barley/wheat/canola (WBC) meal on

performance, N excretion, CO_2 and CH_4 emissions; (3) assess the effect of nutritionally adequate very-low protein diet based on barley plus synthetic amino acids on nitrogen, carbon, energy balance and greenhouse gas emission by growing-finishing pigs; and (4) evaluate the influence of dietary crude protein (CP) and phosphorus reduction with phytase and xylanase, individually or combination, on total tract nutrient digestibility and energy metabolism in growing-finishing pigs. These data will also provide: (1) new estimates of the maintenance energy requirements of current genetic lines sows and grow-finish pigs; (2) basis for the introduction and adoption of the NE system in Canada; (3) estimates of total GHG production (in CO_2-equivalents) from pig production, and (4) quantify the level and effect of enzyme (carbohydrase) inclusion in diet on protein utilization, nitrogen (N) excretion, carbon dioxide (CO_2) and methane (CH_4) excretion by grower-finisher pigs. Also, to assess the potential economic impact of the above interventions since cost-effectiveness is always the ultimate arbitrator in the adoption of any novel feed technology.

However, phytase addition to diets has not been studied under these aspects and no data about its effectiveness in reducing CO_2 or optimal inclusion in diets is available. Addition of cellulases and hemicellulases improve animal performance by degrading non-starch polysaccharides (NSP) that may interfere with digestion of other nutrients (Li et al., 1995). This improvement is primarily in barley/wheat-based diets (Thacker and Baas, 1996), but may also occur in corn-soybean meal diets (NRC, 1998). More importantly, a beneficial effect of the addition of these enzymes should be a reduction in methane (CH_4) production, which is linearly and positively related to the ingestion of non-starch polysaccharides (NSP) (Jensen, 1996). There are currently no data available in the literature to assess the effectiveness of these enzymes for reducing CH_4 emissions, nor about the necessary inclusion levels to achieve this goal.

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3.0 EFFECT OF LOW-PROTEIN DIETS FED TO SOWS AT DIFFERENT STAGES OF THE REPRODUCTIVE CYCLE ON CARBON AND NITROGEN BALANCE

3.1 Introduction

Escalating public awareness about environmental pollutants, notably nitrogen, and phosphorus from pork production has necessitated re-evaluation of traditional feeding and management practices. Experimental evidence indicates that the environmental impact of swine production can be reduced through diet modification (Lenis, 1989; Jongbloed and Lenis, 1992). Emissions of carbon dioxide (CO_2) and to, a lesser extent, methane (CH_4) and nitrous oxide (N_2O) created by bacterial action during the storage and application of manure, contributes considerably to the greenhouse gas emissions (GHG) from agriculture (Mathison et al., 1999). The reduction of nitrogen excretion by the animals appears to lead to a reduction in nitrous oxide emissions from manure (feces and urine) (Misselbrook et al., 1998).

When the dietary protein content is reduced, the diet will contain a larger percentage of carbohydrates and/or fat. Given the greater efficiency of utilization of carbohydrate or fat carbon than protein carbon for body fat synthesis in pigs (van Milgen et al., 2001), reduced protein diets can be expected to lower CO_2 emissions as well as N excretion. Thus, a secondary benefit of reduced dietary protein contents may be a reduction in animals' CO_2 emissions as a result of an improved utilization of dietary energy (Möhn and Susenbeth, 1995). CH_4 emissions appear linearly related to the intake of non-starch polysaccharides (NSP) (Jensen, 1996). Therefore, the cereal on which sow diets are predominantly based, barley in northern and western North America, corn in eastern and central North America, may affect the CH_4 emissions from sows.

There is a dearth of information in the literature on the impact of low-protein diets on sows. To the best of the authors' knowledge, the influence of dietary protein content in sow diets on CO_2 and CH_4 emissions is not known. Direct measurements of the effectiveness of reduced dietary protein on CO_2 emissions are not available. Whether

CO_2 production from pigs can be altered by diet manipulations was not known. Similarly, possible effects of a reduction of dietary protein intake on CH_4 emissions had not been studied. The available literature demonstrates the efficacy of low protein, amino acid supplemented diets in reducing nitrogen excretion by growing pigs (Jongbloed and Lenis, 1992; Kerr and Easter, 1995), however animal performance has been variable. Although mature sows contribute two-thirds of the nitrogen excreted by swine population (Mathison et al., 1999), the effects of feeding low protein, amino acid supplemented diets on sow performance has not been thoroughly examined.

In view of the dearth of knowledge of the suitability of low protein diets for sows, and the differences between growing-finishing pigs and sows at different physiological stages, research is required to generate benchmarks estimating sow performance with low protein feeding and gaseous emissions by sows. It was hypothesized that feeding properly formulated reduced-protein amino acid supplemented diets would reduce CO_2 and CH_4 emission. The objectives of this study were to determine: 1) the effect of diet ingredients and protein level on CO_2 and CH_4 production, 2) the excretion of nitrogen and carbon by sows, and 3) use the data as a basis for calculation of greenhouse gas emissions (CO_2-equivalents) from sows.

3.2 Materials and Methods

All procedures were approved by the Faculty Animal Policy and Welfare Committee for ensuring adherence to the Canadian Council of Animal Care (CCAC, 1993) guidelines.

3.2.1 Experimental Designs

Experiment 1(Non-pregnant sows fed during maintenance): Effect of protein level and diet composition on sows at maintenance. Four fourth parity Landrace X Large White sows (237.8 kg ± 2.5 kg) from the University of Alberta's Swine Research Unit were selected. The effect of dietary protein reduction on CO_2 and CH_4 production, and O_2 consumption was determined in the non-pregnant sows in a 4 X 4 Latin square with two levels of protein, HP versus LP (13.6 vs. 11.1%, CP in the barley/canola; and 12.1 vs. 9.2%, CP in the corn/soybean meal diets, respectively), and two diet types (barley/canola

vs. corn/soybean), as in Tables 3.1a and 3.2a. Each animal was an experimental unit. The four sows were adapted to each experimental diet for at least one week, before indirect calorimetry was performed twice per diet to measure gas exchange. *Experiment 2(Sows fed during gestation and lactation):* Effect of low protein diet for sows at different stages of the reproductive cycle on CO_2 and CH_4 emission. Eighty second parity Landrace X Large White sows (164.3 kg ± 2.1 kg) from the Swine Research Unit of University of Alberta were allocated in 11 groups to a 2 X 2 X 2 factorial arrangement of treatments, as shown in Figure 1. Sows were allocated in pairs immediately after breeding to either a commercial style high protein diet (HP), or low protein (LP) diet supplemented with synthetic amino acids (L-lysine HCl and L-threonine, Degussa, AG). Sow allocation continued until 40 sows per treatment had been confirmed pregnant via ultrasound testing.

3.2.2 Diet Composition

Experiment 1(Non-pregnant sows fed during maintenance): Four diets were formulated based on barley-canola meal or corn-soybean meal (Table 3.1a) for non-pregnant sows fed during maintenance. These diets differed in non-starch polysaccharide (NSP) content and, therefore, the potential to cause CH_4 emissions. In two further diets, the canola or soybean meal inclusion was minimized by supplementing with commercially available feed-grade amino acids (L-lysine HCl, L-threonine, DL-methionine and L-tryptophan, Degussa, AG) to achieve diets hypothesized to decrease both N excretion and C emission (Table 3.1b). The HP diet was the standard gestation diet at the Swine Research Unit, University of Alberta (Aherne and Foxcroft, 2000). Nutrient levels in this diet served as reference for the LP diet, formulated to be approximately 20% lower in crude protein, and isoenergetic to the HP diet. The LP diet was formulated to the same true ileal digestible amino acid content as found in the HP diet. In all diets, the concentrations of all indispensable amino acids, minerals and vitamins met or exceeded the NRC (1998) recommendations.

Experiment 2(Sows fed during gestation and lactation): Diets were formulated based on wheat and barley (Table 3.1c). The conventional diets (HP) were the standard gestation

and lactation diets formulated specifically for the genetic stock at the University of Alberta's Swine Research Unit. Nutrient levels in these diets were used as the reference for the low protein amino acid-supplemented diets (LP), described by McMillan (2003). In all diets, the concentration of all indispensable amino acids (IAA), minerals and vitamins met or exceeded the NRC (1998) recommendations.

Feed allowance was re-evaluated at early gestation (d 35 to 45) and late gestation (d 95 to 105), based on body weight and back-fat at the time and, if necessary, readjusted to meet the target back-fat depth of 20 mm at farrowing. Individual feed intake was recorded daily; orts were weighed daily and subtracted from that animals' recorded intake.

The standard lactation diet at the Swine Research Unit, University of Alberta was used as the HP diet (Table 3.1c). The LP diet was formulated to be approximately 20% lower in crude protein, and iso-energetic to the HP diet. Both diets were composed of mainly wheat, barley and soybean meal. The protein content in the low protein diet was decreased by reducing the inclusion of soybean meal in the LP diet. To maintain the contents of the branched-chain amino acids, without very expensive supplementation, blood meal was included in the diets at a rate of 2.3%.

3.2.3 Housing and Feeding

In Exp. 1(*Non-pregnant sows fed during maintenance*), the non-pregnant sows were individually housed at the Metabolic Unit, University of Alberta research station. Each animal received all 4 diets and served as their own control. The sows were adapted to each diet for at least 7 d. The sows were fed twice daily to provide energy at 10% above calculated maintenance requirement (NRC, 1998) during the adaptation period. Following adaptation, two 4 h respiration measurements were performed on d 7 and 9, separated by a rest period of 1 week. Water was available ad libitum via low pressure nipple drinkers.

In Exp. 2 (*Sows fed during gestation and lactation*), the gestating and lactating sows were housed in open pens (as breeding groups) with individual stalls for feeding, or in

gestation crates. During gestation, sows were fed restrictively (once daily in the morning). Sows received 2.0 to 2.5 kg per day when in second parity, and 2.0 to 3.0 kg when in third parity. Daily feed allowance was calculated based on weight and back-fat depth at breeding, to achieve sufficient body reserves for the following lactation (Aherne and Foxcroft, 2000). Sows were moved into farrowing crates on d 109 of gestation and switched to a 3.0 kg daily ration of lactation diet until farrowing. Orts were weighed daily and subtracted from the daily allowance to account for the recorded daily intake per sow. Upon farrowing feed offered was increased by 0.5 kg per day until sows were eating 5.0 kg and then was increased by 1.0 kg per day until maximum feed intake was reached. Feed was offered once daily in the morning until farrowing and then three times daily (0700, 1200 and 1530) ensuring that feed was available ad libitum to the lactating sow. Temperature inside the gestation and lactation units were maintained as close to 18 to 20°C as possible. If the winter overnight temperature in the gestation area dropped below 18°C, feed allowance of all sows, for the following day, was increased by 50 g for each degree drop. Water was available ad libitum to sows and piglets via two low pressure nipple drinkers.

3.2.4 Growth Measurements

Body weights of the sows (in both experiments) were measured weekly. Gestation sows were also weighed at breeding, early (d 35 to 45) and late gestation (d 95 to 105), and at approximately d 109 of gestation, to the nearest kilogram on a scale (Accurate Scale Industries, Ltd., Edmonton, AB) with a digital readout (DF 2000, Massload Technologies, Saskatoon, SK). Back-fat depth measurements on gestating sows were taken ultrasonically (Scanoprobe, Scanco Inc., Ithaca, NY) at the same specific intervals at the last rib, 65 mm from the midline on both sides during the experiment.

3.2.5 Respiration Measurements

The respiration chambers (234.5 cm x 86 cm x 106 cm per chamber) consisted of commercial farrowing crates enclosed in plexiglass boxes, equipped with a feeder, low pressure nipple drinker, and a cooler to maintain temperature in the thermoneutral zone. Air was drawn through these boxes via an inlet at the lower rear and an outlet above the

feed trough at rates of approximately 240 to 250 L/min. Air flow was measured, after passing through a cold water condenser, with commercial air meters (Model 0543, Canadian Meter Corp., Cambridge, Canada). A sample of air was drawn with a small air pump (Gast Model 0531, Gast Mfg. Corp., Benton Harbour, MI) and delivered to O_2 (Taylor-Servomex, Crowborough, UK) and CO_2 analyzers (Beckman LB2, Beckman, Irvine, CA). Air flow to the analyzers was regulated to 0.5 L/min by ball-type flow meters (Scienceware Size 2, Fisher Scientific, Mississauga, Canada). The analog output (mV) of the analyzers was converted to digital data by an analog-digital converter (Data grabber, Data Electronics, Australia) and recorded by a computer. Another system comprising of O_2, CO_2 and CH_4 analyzers (Qubit System, Kingston, ON) was connected in series to O_2 (Taylor-Servomex, Crowborough, UK) and CO_2 analyzers (Beckman LB2, Beckman, Irvine, CA) analyzers to incorporate CH_4 data acquisition. Data acquisition was set for maximum rate (four readings per second and the average gas concentration for each minute was recorded. Prior to start of respiration studies analyzers were calibrated for zero and gain readings with either pure N_2 (zero) or span gas (1% CO_2, 20% O_2, and 79% N_2). Measurements of these gases at steady state and room air were recorded before and after the test period.

Measurements were continuously recorded for four hours and animals were fed one quarter of their daily ration every hour. In Exp. 1 the respiration study involving the non-pregnant sows consisted of 1 h equilibration period plus 4 h test period. During this period the non-pregnant sows were confined in the respiration chambers and expired air was analyzed continuously for O_2, CO_2 and CH_4. In Exp. 2, expired air was analyzed continuously for O_2 and CO_2 in gestating and lactating sows; the CH_4 analyzer was not available during this period. Lactating sows and their litters were treated as a single entity for respiration measurements. Procedures for respiration measurements were identical to that for gestating sows, except that air flow was increased to approximately 310 L/min to account for the extra CO_2 emission from the lactating sow and its litter. Visual observations of nursing and piglet activity were recorded. Lactating sows tended not to eat at every hourly feeding, so feeding behaviour, at the time when it occurred, was also recorded. In addition to the four hours of respiration with piglets, the sows were

measured alone for 40 min following removal of the piglets. Separation of the sow and the litter for this short period did not appear to cause stress or discomfort to the sows.

3.2.6 Chemical Analyses

Feed, fecal and urine samples were collected and stored in a -20°C freezer prior to chemical analyses. Feed samples from each experiment were taken weekly, and pooled within diet type. Prior to proximate analyses, feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a 1.0 mm screen. Pooled feed samples were analyzed for amino acids by Degussa AG, (Hanau, Germany) using ion exchange chromatography (Llames and Fontaine, 1994). Tryptophan was analyzed by fluorescence detection HPLC after alkaline hydrolysis with barium hydroxide octahydrate at 110°C for 20 h (Fontaine et al., 1998). Gross energy of feed and fecal samples was determined using an adiabatic bomb calorimeter (Leco AC-300, LECO Corp., St. Joseph, MI). Feed, fecal and urine N content were determined by micro-Kjeldahl (AOAC, 1990). Proximate analyses involving dry matter, crude protein (macro-Kjeldahl, AOAC, 1990), ash, ether extract (Goldfisch extraction apparatus, Labconco Corp., Kansas City, MO), neutral detergent fiber , acid detergent fiber, and acid detergent lignin (Ankom Fiber Technique, Goering and van Soest, 1970) were determined on feed and fecal samples. Total carbon content of feed and freeze-dried fecal sample ground in a MM2 Retsch/Birkmann mixer mill (Brinkmann Instruments Inc., Westbury, NY), were analyzed using a NA1500 Carlo-Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ).

3.2.7 Calculations

Heat production in sows at maintenance was calculated using the formula:

$HE\ (kJ) = 16.18 * V_{O2} + 5.02 * V_{CO2} - 5.99 * U_N - 2.17 * V_{CH4}$ (Brouwer, 1965),

where HE is heat production, V is the volume of gas in liters, and U_N is nitrogen excreted into urine, in grams. Heat production in gestating and lactating sows was calculated using a modified formula of Brouwer (1965). The respiratory quotient was calculated according to the equation: $RQ = V_{CO2}/ V_{O2}$ to determine the types of substrate utilized by the sow during the measurement period. CO_2-equivalents, from the sows, were calculated using

factors of the global warming potential (GWP): 1, 21 and 310 for CO_2, CH_4 and N_2O respectively, on a molar basis (Grubb et al., 1996; IPCC, 1997). The conversion of nutrients in manure was not known; therefore the CO_2-equivalents from manure were calculated using a range in estimated conversion rates of both 5 and 30% of the N in manure to N_2O (Béline et al., 1999) and 3 and 20% of C in manure to CH_4 (Martinez et al., 1999).

In the gestating and lactating sows because total nitrogen excretion or methane emission was not measured, the Brouwer formula was reduced to:

$$HE\ (kJ) = 16.18 * V_{O2} + 5.02 * V_{CO2}.$$

With the removal of the methane and nitrogen excretion terms, the reduced formula results in an overestimation of HE by approximately 1.5%. The respiratory quotient was, however calculated according to the same equation: $RQ = V_{CO2}/ V_{O2}$ to determine the types of substrate utilized by the sow during the measurement period

3.2.8 Statistical Analyses

In Exp. 1, the effects of type of diet and protein level were estimated using the mixed model procedures (SAS, 1999). The interaction between type of diet and protein level was tested for all outcome parameters, and used to calculate least square means (LSM) and the probability of differences between treatments. In Exp. 2, a multi-way factorial approach comparing two diet regimens (HP and LP) during two parities (second and third) with two time points per reproductive stage (early and late) was used. Analysis of variance was performed using mixed model procedures (SAS, 1999) with 'sow' as a random variable. Interaction of factors were retained in the model if $P < 0.1$. Group least square means were compared using the 'pdiff' option, which performs a pair-wise comparison based on a two-tailed t-test. Significance in both experiments was determined as $P < 0.05$, and P-values between 0.05 and 0.1 were regarded as trends.

3.3 Results

In Exp. 1, non-pregnant sows (n = 4, weighing 237.8 ± 2.5 kg), consumed more (P = 0.001) of the barley diet than the corn diet because they were fed to equivalent energy

intake. Within each ingredient type, consumption was not different ($P = 0.58$) between the high protein (HP) and low protein (LP) diets. The protein intake was greater for the barley diets ($P = 0.001$) and for the HP diets ($P = 0.001$). The carbon intake was greater for the barley diets ($P = 0.001$), but not different ($P = 0.71$) between protein levels (Table 3.3a). The CO_2 production by non-pregnant sows increased with feed intake (Table 3.4). The CO_2 production tended to be greater ($P = 0.08$) for LP than HP by 5% and 6% for the barley-canola and corn-soybean based diets respectively, and, when using energy intake as a covariate was not different ($P = 0.26$) between types of ingredient. The CH_4 production was lower ($P = 0.001$) for LP when feeding barley-canola based diets, but similar for LP and HP when feeding corn-soybean based diets. The CH_4 production was lower ($P = 0.03$) for the corn-soybean meal than for barley-canola based diets. Overall, the CH_4 production was related to the intake of acid detergent fibre (ADF), ($r = 0.62$; $P = 0.001$). The CO_2-equivalents directly produced by the sow is represented by the sum of CO_2 and CH_4 production, applying the GWP factors for the gases. This was reduced ($P = 0.04$) by 19% for LP when feeding barley-canola based, but increased ($P = 0.11$) by 11% for the corn-soybean meal based diets, resulting in an interaction between type of diet and protein level. Overall, the 6.3% reduction in CO_2-equivalents by dietary protein concentration did not reach significance ($P = 0.16$; Table 3.4).

In Exp. 2, there were distinct feeding levels for gestation and lactation. The effect of feed intake could not be separated from diet for the combined data for gestating and lactating sows. In order to evaluate the effect of diet, separately from feed intake, on CO_2 production, data was separated into gestation and lactation for analyses. This also allowed for the evaluation of piglet effect on energy expenditure. The main effects evaluated were diet, parity and stage, with feed intake used as covariate for both gestation and lactation.

The daily feed intake of gestating sows did not differ ($P > 0.1$) between protein levels, but was greater ($P = 0.001$) in parity three than two (Table 3.3b). The protein intake was lower by 18% ($P = 0.001$) sows fed LP diet, while the carbon intake was lower by 5.7% ($P = 0.001$). Both protein and carbon intake were greater ($P = 0.001$) in parity three than two. The reduction in dietary protein concentration did not affect sow performance

during gestation. The total and maternal weight gains of sows was not different ($P > 0.1$) between high and low protein diets (McMillan, 2003). In parity three, total and maternal weight gains were greater ($P < 0.007$) than in two. The reduction in dietary protein concentration did not impact sow performance during gestation, but led to reduction in both intake and excretion of nitrogen and carbon.

3.3.1 CO_2 Production by Gestating and Lactating Sows

The experiment studying the effects of reducing dietary protein on gestating and lactating sows was conducted prior to the acquisition of the methane analyzer; therefore only the CO_2 production was measured. For sows fed LP diet during gestation, CO_2 production was lower 5.4 %, compared to sows fed the HP diet (Table 3.7b). Stage of gestation significantly affected CO_2 production. Sows produced less CO_2 during early gestation (d 45) compared to late gestation (d 95) ($P = 0.009$, Table 3.7b). Regardless of dietary treatment, CO_2 production by the sows was lower ($P < 0.001$) in the second than third parity. There was a significant interaction ($P = 0.05$) between diet and parity on production of CO_2 by gestating sows (Data not shown). For both dietary treatments, CO_2 production increased with increasing parity. CO_2 production was similar for the two dietary treatments during parity two ($P > 0.05$), but higher ($P < 0.05$) for HP fed sows during parity three than two (Data not shown).

Lactating sows offered the LP consumed less ($P < 0.005$, Table 3.3b) feed, protein, and carbon per day than sows offered HP. Feed, protein and carbon intake were greater ($P < 0.005$) in third than second parity (Table 3.3b). McMillan (2003) reported that feed intake of all the lactating sows in this experiment was insufficient to maintain body weight and back-fat thickness; however, sows in parity three lost less body weight than in two. The number of piglets born alive was not different between dietary treatments. The daily milk production per piglet was not different between dietary treatments. The mean birth weight per piglet was non-significantly lower for the LP treatment. The number of piglets weaned tended ($P = 0.1$) to be lower for the LP treatment, and the weaned piglets were non-significantly smaller (McMillan, 2003). Overall, the consistent direction of change in performance of sows and their litter suggest that these may be lower when fed LP

compared to HP diets. Although the diets conformed to the recommendations for nutrient concentration (NRC, 1998) these data suggest that these may be slightly incorrect for the LP sows. Additional research is required to clarify this issue.

The gas exchange of lactating sows without litter was measured to account for effect of piglets on gas measurements (Table 3.7b). During a 40 min period whilst sows (without litter) were confined to the respiration boxes, CO_2 production was not significantly affected by dietary treatment, although the CO_2 production from LP fed sows was lower by 3.9% (Table 3.7b). Early (d 6) or late (d 16) stage of lactation did not influence CO_2 production ($P > 0.1$). Parity influenced CO_2 production; sow exhibited lower ($P = 0.001$) CO_2 production during second than third parity (Table 3.7b). CO_2 production was calculated for sow and her litter (Table 3.7b). The data were adjusted using feed intake and nursing piglets as covariates. LP fed sows and their litter showed a trend ($P = 0.1$) to produce 2.5% less CO_2 (Table 3.7b). CO_2 production was affected by parity ($P = 0.007$), with sows emitting greater quantities of CO_2 in the third parity (Table 3.7b).

3.3.2 GHG Production by Sows

The CO_2 production of sows fed LP diet was 6% greater for non-pregnant sows during maintenance, whilst it was between 2.5 and 5.4% lower for sows during gestation and lactation (Table 3.9). Typically, sows spend about one week out of 5 months neither pregnant nor lactating, thus it can be concluded that a reduction of dietary protein contents will reduce CO_2 production. In Exp.1, the CH_4 emission from non-pregnant sows was found to be 0.821 g/MJ metabolizable energy intake for the HP, and 0.558 g/MJ metabolizable energy intake for the LP diet. Yearly estimated CO_2-equivalents (Table 9) were based on the following assumptions: gestation period of 115 d, 7 d return to oestrus, 9 d for 20% of the sow herd not inseminated successfully at the first oestrus after weaning, and duration of lactation as 23 d. These assumptions calculate to154 d per parity or 2.37 parities per year. Therefore, the CO_2-equivalents by sow and year are 2015 kg for HP, and 1682.4 kg for LP fed sow, a reduction of 16.6% (Table 3.9). Thus, a 20% reduction in dietary protein content was estimated to reduce CO_2-equivalents emitted per sow per year by 333.5 kg (from a population of 1000 sows).

3.4 Discussion

Most parameters of sow performance before allocation were similar for the subsequent LP or HP treatments (Data not shown). However, sows allocated subsequently to the LP treatment had smaller piglets at birth and lower feed intake despite longer lactation compared to sows fed the HP diet (McMillan, 2003).

Feed and nutrient intake for LP sows was lower than that for HP sows, and lower in parity 2 than in parity 3 (McMillan, 2003). Feed and nutrient intake after imposition of dietary treatments increased with increasing first parity lactation feed intake (McMillan, 2003). Sow body condition was not different between sows fed HP and LP diets, or between parity 2 and 3, except for greater body weight in HP-fed than LP-fed sows, and in parity 3 than 2. Increasing lactation feed intake increased body weight and backfat thickness at weaning and reduced body weight and backfat loss. Increasing litter size had the opposite effect. Increasing age at first service increased body weight and backfat thickness at farrowing and weaning. Increased gestation feed intake decreased backfat thickness at farrowing and weaning. Increasing backfat thickness at farrowing increased backfat loss during lactation.

Gross energy and N digestibility were greater for HP-fed than for LP-fed sows, but not affected by parity. Gross energy and N digestibility decreased with increasing feed intake. N digestibility was lower in late than early lactation. The N to creatinine ratio was reduced by 28.6% in LP-fed compared to HP-fed sows, and lower for parity 2 than parity 3. Stage of lactation did not affect the N to creatinine ratio. Estimated N excretion was reduced by 31% in LP-fed compared to HP-fed sows, but not affected by parity.

HE of sows with litters was greater for HP than LP, and greater in late than early lactation, but not affected by parity. HE increased with feed intake and litter size. Measured HE of sows alone tended to be greater for HP-fed compared to LP-fed sows, and greater in parity 3 compared to parity 2, but not affected by the stage of lactation. Heat production of sows alone increased with feed intake. The respiratory quotient (RQ) was similar for LP-fed and HP-fed sows, greater in parity 3 than parity 2, snd greater for

late than early lactation. The RQ increased with feed intake increased with feed intake. When piglets were removed, the same effects were found except that stage of lactation was not a significant factor.

Diets reduced in CP and supplemented with free amino acids had no impact on most performance parameters for lactating sows. However, nutrient intake, piglet growth rate and and piglet weaning weight were reduced for sows fed the LP diet. These results need to be discussed with respect to impact of genetic and management considerations. Sows on both dietary treatments lost body weight and backfat during lactation. Post-fact analysis showed that LP-fed sows had reduced feed intake prior to allocation of treatment. It is therefore possible that the reduction of feed intake in LP-fed sows was genetically caused rather than an effect of the dietary treatment, especially as feed intake in growing-finishing pigs is moderately heritable ($h^2 = 0.3$, Webb, 1989). Using feed intake as a covariate showed that dietary treatment had no effect on sow body weight nor backfat change during lactation, which is in agreement with previous studies of low protein diets for sows (Falaschini et al., 1994; Johnston et al., 1999; Renaudeau et al., 2001; Trombetta et al., 2001). Reducing dietary CP did not affect the number of piglets born alive nor weaned, similar to observations from other studies examining LP diets (O'Grady and Hanrahan, 1975; Corley et al., 1983; Falaschini et al., 1994; Renaudeau and Noblet, 2001).

The lower energy and protein digestibilities in LP compared to HP may have been caused by the higher fiber (NDF) content in LP. Wenk (2001) and Noblet and Perez (1993) showed that increased dietary fiber negatively affects energy and protein digestibilities respectively, in growing pigs. However, Cuaron et al. (1984) and Kerr and Easter (1986) reported no difference in protein digestibility in sows fed LP, amino acid-supplemented or conventional diets. The reduction of digestibility at increased feed intake was in agreement with the general observation that a negative relationship between feed intake and diet digestibility (Gomez et al., 2002). The reduction in dietary protein by 2.8 pu or 14% (LP relative to HP) reduced N excretion by 28.6% based on the urinary N to creatinine ratio, and by 31% based on the difference between digestible protein intake

and N retention estimated according to NRC (1998). This is somewhat greater than the conservative (consensual) estimate of 8% reduction in N excretion for each 1% dietary protein reduction in growing-finishing pigs (Jongbloed and Lenis, 1992; Kerr et al., 1995; Sutton et al., 1999; Lee et al., 2001). However, Nonn et al. (1996) achieved a reduction of N excretion in sows by more than one-third when reducing dietary protein by 13% relative to recommendations. It therefore appears that reducing dietary protein may be more effective in sows than in growing-finishing pigs, to reduce N excretion.

The lower DE intake for the LP diet, in this study, was partially compensated by reduced HE from LP-fed sows. The HE decrease of 13.3 kJ/g reduction in protein intake was substantially greater than the estimate 7.0 kJ/g (Bellego et al., 2001) or the 6.8 to 8.4 kJ/g derived from Möhn and Susenbeth (1995). As opposed to growing-finishing pigs, the lactating sows were in a catabolic state, with a non-significant increase in body tissue mobilization (kpf) in LP-fed sows. The increased efficiency of body tissue for milk production compared to oral nutrients may have contributed to the relatively large reduction in HE in the LP-fed sows. In support for this argument, similar RQ for HP-fed and LP-fed sows was observed, which indicates equal utilization of nutrients.

The greater feed and nutrient intake of sows in parity 3 probably prevented greater loss of body weight during lactation despite larger litter size and piglet growth rate than in parity 2. Mahan (1998) reported increased feed intake, litter size and litter growth rate from parity 2 to parity 3, with little difference in body weight change. The increased piglet growth was caused by increased milk production in parity 3, which is in agreement with that reported by Etienne et al. (1998). Milk composition, however, did not change from parity 2 to parity 3 as observed by Klobasa et al. (1987).

Urinary N excretion increased from parity 2 to parity 3, because the daily intake of lysine (the first limiting amino acid) was closer to requirements in parity 3. It appears that greater excess of some amino acids caused the increase in the N to creatinine ratio. HE was not affected by parity, but the RQ was greater for parity 3 than parity 2. This indicates a greater reliance of the sow on feed (dietary) nutrients than body tissue

mobilization (Chwalibog et al., 2003), and is in agreement with reduced body weight losses in parity 3 (McMillan, 2003).

It appears that the first parity performance of sows determines, in part, the performance in subsequent parities. This applies especially to lactation feed intake, but also to the number of piglets born alive and their birth weight and growth rate, which all showed a significant positive correlation between first and later parities. Piglets with greater birth weight, in turn, had greater growth rate and weaning weight, and were less likely to die. Conversely, larger litters had smaller piglets with lower growth rate.

There appears to be no carry-over effects of gestation dietary treatment on lactation performance of either sow or piglets. Cooper et al. (2003) reported an impact of gestation lysine intake on litter weight for first parity, but not for parities 2 and 3. Gestation feed allowance (intake) is a management decision; sows in poorer conditions were allocated greater feed allowance (Aherne and Foxcroft, 2000).

The composition of milk has been shown to change as lactation progresses (Klobasa et a., 1987; Sauber et al., 1998; Jones and Stahly, 1999). Klobasa et al. (1987) observed a decline in milk protein concentration from day 2 to 3 weeks after farrowing, while total solids and fat content remained relatively stable during this period. This agrees with the findings, where milk protein decreased from early to late lactation, and dry matter (DM), fat and gross energy contents did not change significantly (McMillan, 2003). Decreases in the concentrations of milk DM, fat, GE and protein, as lactation progressed have been reported (Sauber et al., 1998; Jones and Stahly, 1999). However, these studies manipulated both dietary protein and amino acid supplies. The stability of milk fat concentration between days 7.5 and 16.2 of lactation is likely a function of the direct transfer of lipids from the blood to milk, whereas protein in the milk must be synthesized in the mammary gland.

The increase in relative N excretion from parity 2 to 3 reflected the increase in feed allowance, and thus amino acid intake during parity 3. More likely, sows consumed

greater excess of amino acids relative to their requirements during parity 3, but consumed sufficient amino acids to meet their requirements during both parities. Interestingly, the RQ increased from parity 2 to 3 (McMillan, 2003). The lower RQ during parity 2 indicated that sows were utilizing more fat during this period than in parity 3, which agrees (concurs) with the greater fat loss of lactating sows during parity 2, and the backfat loss observed during late gestation same parity (parity 2) (McMillan, 2003). Since more O_2 was consumed (utilized) during the oxidation of fat compared to carbohydrate oxidation, the increase in RQ during gestation from parity 2 to 3 (McMillan, 2003) indicates that parity 2 sows were utilizing fat to supply energy, while during parity 3 more carbohydrates were utilized to supply energy. The lack of parity interactions on measured sow performance traits indicated that feeding the LP, amino acid-supplemented diets during parity 2 did not affect performance in parity 3. This observation supports one of the hypotheses, that feeding LP diets in a previous parity does not impact sow performance in the next parity (McMillan, 2003).

Heat production can be measured directly by physical methods or indirectly, through measurements of gas exchange in respiration chambers, or through calculations of nitrogen and carbon balance (McLean and Tobin, 1987). Balance studies measure the amount of carbon and nitrogen ingested, excreted, and retained in the animal. To accurately determine heat production, gaseous excretion of carbon (as CO_2 and CH_4) must also be measured. Direct calorimetry measures the rate of heat dissipation as determined by changes in temperature and humidity, whereas indirect calorimetry measures the rate of heat generation calculated by gas consumption and production. Alternatively, indirect calorimetry can be performed using a close-circuit (air-tight) or open-circuit (constant air flow) systems. The open circuit respiration system, equipped with monitors to measure oxygen and carbon dioxide concentrations in respired and ambient air, is the most common method of measuring gas exchange in large animal studies. As well, respiratory analysis of oxygen and carbon dioxide provides a reasonable estimate of heat production (McLean and Tobin, 1987), which can be calculated using the formula of Brouwer (1965):

$$HE\ (kJ) = 16.18*V_{O2} + 5.02*V_{CO2} - 5.99*N - 2.17*VCH_4,$$

where HE is heat production (energy), V is volume of gas in liters, and N is nitrogen excreted.

To the author's knowledge, energy expenditure of pregnant and lactating sows fed LP, amino acid-supplemented diets, by indirect calorimetry has not been previously reported. Furthermore, very few studies have examined the effects of physiological state, stage of pregnancy or lactation and parity on energy expenditure (Close et al., 1985; Noblet and Etienne, 1987), but none have examined these under LP feeding regimens. The lack of interactions between diet and the other main effects tested, indicated that generally energy expenditure of sows that received both dietary treatments responded similarly to changes in physiological state, stage and parity. With reduced intake of excess amino acids, less of the DE was used to catabolize amino acids, thus increasing the amount of energy available for tissue deposition (Le Bellego et al., 2001) and other metabolic processes. Since a decrease in N excretion was observed, and there is an energy cost associated with N catabolism and excretion, a reduction in HE would be expected. LP-fed sows demonstrated overall reduction in CO_2 emission of 6.6% and reduction in daily HE of 8%, indicating that the LP-fed sows used the available energy more efficiently, over the course of the study. Also, HE per metabolic body weight was lower, supporting the hypothesis that sows fed LP, amino acid-supplemented diets would be energetically more efficient, and would utilize dietary energy to a better extent than HP-fed sows.

The overall RQ was similar between sows fed HP and LP diets, indicating that sows utilized similar proportions of subtrates during the study. The increase in gas exchange and HE from gestation to lactation was expected because sows in lactation have both higher feed intake and increased metabolism compared to pregnant sows. The increase in RQ from pregnancy to lactation was probably an indication of variation in diet composition and feed intake, suggesting that sows in lactation used more of the dietary carbohydrates (CHO) for metabolic processes. Furthermore, the increase in gas exchange and HE with increasing parity was likely due to increased sow feed intake and body weight, and an increase in metabolism caused by larger litter sizes and milk production (McMillan, 2003). When calculated on a metabolic body weight ($BW^{0.75}$) basis, HE

followed an opposite trend, with lower HE per unit of metabolic body weight ($BW^{0.75}$) in parity 3 compared to parity 2. Apparently, because sows were larger in parity three than in parity two, maintenance heat loss accounted for a greater proportion of their HE. Additionally, changes in metabolic body weight are not linear with changes in total body weight; therefore, the large weight gain observed from parity 2 to parity 3 would only equate to a small change in metabolic body weight. Despite the greater body mass of the sows during parity 3, they had lower HE per unit of metabolic body weight than during parity 2. Remarkably, the RQ increased from parity 2 to parity 3. The lower RQ during parity 2 compared to the value in parity 3 suggested that sows were using more fat during this period than in parity 3, in agreement with the greater fat loss in lactating sows in parity 2 and loss of backfat during late gestation of that parity reported by McMillan (2003).

There was a significant interaction between physiological state and parity for gas exchange and HE such that CO_2 emission increased, during gestation and lactation as parity increased. However, O_2 consumption and HE were similar across parities during gestation, but increased from parity 2 to 3 during lactation. The increase O_2 consumption, CO_2 emission and HE during lactation reflected the increases in maternal body weight, feed intake, and litter size in parity 3 (McMillan, 2003). Although the pregnant sows, like the lactating sows, also increased in body mass from parity 2 to parity 3, with a corresponding increase in feed and nutrient intake, only CO_2 emission increased. This suggests that pregnant sows required less O_2 to oxidize the available nutrients, and thus yielded less HE. Noting that more O_2 is used during the oxidation of fat than carbohydrates, the elevated RQ during gestation from parity 2 to 3, indicates that parity 2 sows were using fat to supply energy while carbohydrate was used during parity 3.

The change in HE, expressed on a metabolic body weight basis, with physiological state and stage indicated that overall metabolism of the sow was altered during the reproductive cycle. The decrease in HE per unit of metabolic body weight from early to late gestation indicated that the sow utilized ME more efficiently as pregnancy advanced. This observation is contrary to that of Noblet and Etienne (1987). From energy balances

(EB), Noblet and Etienne (1987) calculated that a 160 kg sow gaining 0.65 kg/d would yield an increase in HE of 0.53 $kJ/kg^{0.75}$, for each day of pregnancy, as a result of increased maintenance energy requirements. However, it is unlikely that this magnitude of increase would be detectable by calorimetry equipment. During early gestation, the sow's own tissues are the main influence on metabolism, and maternal growth comprises a larger portion of weight gain. As pregnancy advances, especially from 90 d, foetal growth becomes considerable (Boyd et al., 2000). The reduction in HE per unit of metabolic body weight from early to late gestation suggests that foetal tissue deposition utilizes available energy more efficiently than maternal tissue deposition. A proportion of maternal tissue gain is fat, to replace that lost during lactation, whereas newborn piglets (neonates) contain very little adipose stores (Tilton et al., 1999), and energy cost for fat deposition is considerably higher than that for lean tissue deposition (Mohn and Susenbeth, 1995; NRC, 1998).

Calorimetry measurements were partitioned into gestation and lactation to account for differences in feed intake, and enable us to measure piglet influences on respiration. Pregnant sows fed low protein, amino acid-supplemented diet were more energy efficient than their conventionally-fed counterparts, as indicated by their reduced gas exchanges and daily HE. Also, HE per unit of metabolic body weight of sows fed the LP diet was reduced. Increasing CO_2 emission with increasing stage of gestation was likely due to the increase in body weight, and greater influence of foetal and mammary tissues on metabolism, from the beginning of gestation to late gestation. Foetal growth is rapid during the last trimester (Noblet et al., 1997; Boyd et al., 2000), and such tissues are metabolically highly active. Additionally, mammary protein deposition increases three fold during late gestation (Boyd et al., 2000) and becomes increasingly metabolically active, in preparation for lactation (Noblet et al., 1997). Alternatively, the increase in CO_2 emission without an increase in O_2 consumption may be reflective of the loss of backfat observed during late gestation of parity 2 (McMillan). Oxidation of body fat would increase CO_2 emission, without increasing O_2 consumption, and would reduce RQ. The lack of increase in heat production, with increasing stage of gestation is in opposition to

Close et al. (1985) and Noblet and Etienne (1987), who both observed increased heat production as gestation progressed, using direct and indirect calorimetry, respectively.

In the study of Close et al. (1985), sows exhibiting increased metabolism were in negative energy balance during late gestation, which would have increased HE; however, in the study of Noblet and Etienne (1987), sows were in positive energy balance. An increase in HE with the advancement of pregnancy may be caused by variations in metabolic body size, maintenance energy requirements and retained energy (RE), under constant feed intake (Noblet and Etienne, 1987). It is possible that any combination of these factors would cancel out the effect of increasing day of gestation on heat production.

Similar to the effect of stage of gestation on gas exchange, the increase in CO_2 emission, but not in oxygen consumption or heat production during gestation, with increasing parity may reflect the mobilization of body fat during parity 2, but not parity 3. This agrees with the RQ data, which indicated that parity 2 sows utilized more fat, and parity 3 sows more carbohydrates as their main energy source. Alternatively, increased carbon dioxide emission is a reflection of increased body weight, and foetal demand, due to greater number of piglets born in parity 3.

The observed interaction between diet and parity on carbon dioxide emission during gestation further supports the N excretion and amino acid intake results, which indicated that parity 2 sows had a higher requirement for amino acids than parity 3. Because feed intake was altered to match body weight changes, it was used as a covariate for statistical analysis of energy expenditure measurements. Therefore, parity differences in CO_2 emission were due to differences in dietary treatment and metabolic function, and not simply feed intake. Gestation sows from HP and LP emitted similar amounts of CO_2 during the second parity, which was lower than the CO_2 emission in parity 3. The increase in CO_2 emission with increasing parity was more pronounced in HP sows, resulting in sows fed the HP diet producing more CO_2 than LP sows during the third parity gestation. The similarity in CO_2 emission for the two dietary treatments during the

second parity suggested that a limitation of energy and/or amino acid might have occurred during this period, to negate the positive effects of protein reduction on CO_2 emission. The sows fed the LP diet exhibited a smaller increase in CO_2 emission compared to HP sows from parity 2 to parity 3 suggested that LP sows altered their metabolism during the third parity gestation. Alternatively, sows fed the LP, amino acid-supplemented diet entered the third parity gestation at a lower weight than HP sows, but proceeded to regain weight faster than HP sows, such that body weight was similar by the early gestation calorimetry measurements, and because backfat depth was similar between the two dietary treatments at all times, we can assume that the increased gain was largely protein (McMillan, 2003). The energy cost associated with deposition of muscle protein is less than that associated with deposition of body fat, and this may have been reflected in the lower CO_2 emission and increased efficiency of LP sows during parity 3.

A reduction in O_2 consumption and heat production in lactating sows fed LP diets compared to sows fed the HP diet was observed, which supports the hypothesis that feeding LP, amino acid-supplemented diets will result in sows that are more energy efficient. Sows fed the LP diet emitted nearly 4% less CO_2 and almost 5% less heat on metabolic body weight basis. The larger body weight loss of LP sows during lactation compared to HP sows with similar backfat loss, was a result of increased lean tissue mobilization and catabolism, which would be expected to increase CO_2 emission and heat production of sows fed the low protein diet, abolishing the possible benefits of reduced excess amino acid supply reported by McMillan (2003). Furthermore, sows were alone for only 40 minutes, and activity level was greatly different (standing vs. lying down) between animals, resulting in a larger standard error. RQ values for early and late lactation suggest that the proportion of substrates used for energy was also similar. The increase in CO_2 emission with increasing parity was likely related to the increases in body mass of the sows and number of piglets nursing the sow from parity 2 to parity 3 (McMillan, 2003). Larger litters cause the sow to produce more milk, which increases the metabolic demands on the sow (Auldist et al., 1998), and thus HE.

Comparable to the measurements during pregnancy, it was observed greater losses of body weight and backfat during lactation of the parity 2, which may explain the lack of difference in O_2 consumption between the two parities. As in pregnancy, the greater fat mobilization in parity 2 lactation would increase O_2 consumption, negating the differences in O_2 consumption caused by lower body weight during parity 2. This increased mobilization of fat was also reflected in the lower RQ observed during parity 2.

As discussed previously, because piglets were removed from the sow for a portion of the respiration measurement, it was possible to obtain separate gas exchange and HE data for the sow alone and sow with its litter. The increase in gas exchange and daily HE between the sow alone, and sow with its litter, can be assumed to be the result of piglet respiration. The effect of dietary treatment on gas exchange and heat production were the same for the sow alone, and sow with its litter (adjusted for feed intake as a covariate) indicating that the decrease in O_2 consumption and heat production observed was a result of the dietary treatment influencing the metabolism of the sow, and not the litter. The gas exchange of the sow and litter, and daily heat produced per piglet increased as lactation progressed, mainly due to the large increase in piglet body weight during lactation. HE of the piglet, expressed relative to metabolic weight, did not change with increasing age.McMillan (2003) reported that the increase in CO_2 emission with increasing parity was likely a result of both increased body weight of the sow, and increased milk production, a trend towards increased HE from the piglet, and increased HE per unit of metabolic body weight of the piglet may have been an indicator of differences in piglet body composition between the parities, which could have resulted from the differences in milk yield and nutrient output (McMillan, 2003).

Like N excretion, the environmental impact of reduced CO_2 emission is worth considering. In this study, HP-fed pregnant sows emitted 3024.2 g/d CO_2 compared to 2861.2 g/d emitted from sows fed the LP diet. Over a 115-day gestation, this would result in a reduction in CO_2 emission of 18.7 kg. Lactating sows fed the HP diet emitted 4535 g/d CO_2, whereas sows fed the LP diet emitted 4359.6 g/d CO_2. This would amount to a reduction of 3.7 kg CO_2 during a 21-d lactation. Therefore, the use of LP diets during

gestation and lactation would potentially reduce CO_2 emission, over the course of one reproductive cycle by approximately 22.4 kg per sow. Extrapolating this to a typical 1000-sow herd achieving 2.3 reproductive cycles per year results in a reduction of 51 T CO_2 per year.

Additionally, the cost benefits of reduced nitrogen and carbon dioxide emission must be considered when utilizing low protein, amino acid-supplemented diets. In future, producers may be able to profit from the reduction in greenhouse gas emissions of their pigs, through the sale of carbon credits. Currently, carbon credits are valued at $15 to S60 per tonne (Ball and Mohn, 2003). In this study, sows fed LP diets had a 32% reduction in GHG (26% reduction in N excretion, 6% reduction in CO_2 emission). Through the reduced GHG, potential savings could be achieved by using LP over HP diets.

3.5 Conclusion

The reduction of protein concentration in diets based on either barley-canola or corn-soybean meal for non-pregnant sows fed during maintenance tended to increase CO_2 emissions. Reduction of dietary protein was associated with lower neutral detergent fiber contents, and thus reduced CH_4 production in non-pregnant sows. The barley-canola LP diet led to a significant reduction in CH_4 production, whilst the CH_4 production was not affected by protein level in the corn-soybean meal diets. The CO_2-equivalents emitted by non-pregnant sows was lower for barley-canola LP diet than barley-canola HP diet. Overall, the CO_2-equivalents were reduced by 16.4% with the reduction of dietary protein concentration.

Reduction of dietary protein concentration for gestating sows had no statistically significant impact on animal performance. However, piglet weight at birth and weaning was non-significantly lower when LP diets were fed to lactating sows. The number of piglets weaned tended to be lower for the LP treatment. The CO_2 production by sows during gestation and lactation (with litter) was lower by 5.4% ($P = 0.01$) and 2.5% ($P = 0.1$), respectively. In the absence of CH_4 measurements, the effect of protein reduction on CH_4 production was assumed to be similar to that from non-pregnant sows fed barley-

canola diets during maintenance. With this assumption, the reduction of dietary protein concentration reduced CO_2-equivalents emitted from sows by 8%. This estimate, however, does not include emissions from manure, which are related to the nutrient contents of manure. Reducing dietary protein concentration had no effect on fecal carbon excretion. The urinary nitrogen excretion, and concomitantly the carbon excretion, was reduced by 26% when sows were fed LP diets. The calculated total nitrogen excretion from sow within a year was reduced by 20%, when LP diets were fed, while carbon excretion was reduced by 6%.

3.6 Implications

Feeding LP amino acid supplemented diets reduced production of CO_2-equivalents by sows due to the lower carbon content in such diets. However, the main effect of reducing the dietary protein content is the reduction of N excretion. These experiments provide baseline information for further research regarding diet formulation for sows. The principles derived from this experiment will be applicable to growing-finishing pigs as well. The implementation of the results of these experiments will aid the reduction of C and N emission from pig production and mitigate public concerns about pollutants into the environment.

Reduced feed and nutrient intake of lactating sows fed a LP, amino acid-supplemented diet reduced milk production and piglet growth rates. After balancing (adjusting) for differences in feed intake and litter (using them as covariates in statistical analysis), the LP diet supported similar milk yields as the conventional (HP) diet. The concentration of protein, fat and energy were lower in the milk of sows fed the LP diets, indicating that the quality of milk was lower in the LP than the HP sows. Interestingly, the amino acid composition of milk appears to be unalterable by dietary manipulation, indicative of highly regulated synthetic pathways. These data indicates that further research to understand the mechanisms of amino acid supply, and litter dynamics on milk production and the milk composition is required. Additionally, it appears that modern sows may be highly sensitive to slight nutritional challenges, and that younger sows will reduce milk production to preserve body tissues.

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Table 3.1a Ingredient (g/kg) composition of diets fed to non-pregnant sows (as fed basis)

Ingredient	Barley-based diet		Corn-based diet	
	High protein	Low protein	High protein	Low protein
Barley	845.0	962.1	0.0	0.0
Corn	0.0	0.0	845.0	935.9
Canola oil	10.0	5.0	0.0	0.0
Soybean meal	0.0	0.0	115.0	25.0
Canola meal	115.0	0.0	0.0	0.0
L-Lysine HCl	0.0	1.9	0.0	2.7
DL-Methionine	0.0	0.0	0.0	0.1
L-Threonine	0.0	1.0	0.0	1.1
L-Tryptophan	0.0	0.0	0.0	0.3
Vitamin-mineral premix[1]	30.0	30.0	35.0	35.0

[1]Provided per kilogram of diet: Ca, 21.5%; available P, 8.4%; Na, 4.8%; Mg, 1%; Cu, 595 mg; I, 9.9 mg; Fe, 7015 mg; Mn, 1600 mg; Se, 7.4 mg; Zn, 3470 mg; vitamin A, 3000000 IU; vitamin D3, 35000 IU; vitamin E, 1550 IU; viatamin K, 41 mg; Biotin, 6 mg; Folacin, 80 mg; Niacin, 950 mg; Pantothenic acid, 625 mg; Riboflavin, 175 mg; vitamin B12, 880 µg.

Table 3.1b Ingredient (g/kg) composition of diets fed to gestating sows (as fed basis)

Ingredient	High protein	Low protein
Wheat	120.0	248.0
Barley	700.0	700.5
Soybean meal	65.0	5.0
Canola meal	65.0	0.0
Tallow	10.0	0.0
L-Lysine HCL	0.0	2.8
L-Threonine	0.0	1.2
Mineral-vitamin premix[1]	40.0	40.0
Dicalcium phosphate	0.0	2.5
Chromic oxide	2.5	2.5

[1]Provided per kilogram of diet: Ca, 21.5%; available P, 8.4%; Na, 4.8%; Mg, 1%; Cu, 595 mg; I, 9.9 mg; Fe, 7015 mg; Mn, 1600 mg; Se, 7.4 mg; Zn, 3470 mg; vitamin A, 3000000 IU; vitamin D3, 35000 IU; vitamin E, 1550 IU; viatamin K, 41 mg; Biotin, 6 mg; Folacin, 80 mg; Niacin, 950 mg; Pantothenic acid, 625 mg; Riboflavin, 175 mg; vitamin B12, 880 µg.

Table 3.1c Ingredient (g/kg) composition of diets fed to lactating sows (as fed basis)

Ingredient	High protein	Low protein
Wheat	510.0	894.1
Barley	200.0	0.0
Soybean meal	225.0	28.0
Tallow	25.0	8.0
L-Lysine HCL	0.0	3.6
L-Threonine	0.0	1.3
Breeder premix[1]	40.0	40.0
Dicalcium phosphate	0.0	2.0
Bloodmeal	0.0	23.0
Chromic oxide	2.5	2.5

[1]Provided per kilogram of diet: Calcium, 8.6 g; Phosphorus, 3.4 g; Sodium, 1.9 g; Mgnesium, 0.4 g; Sulphur, 80 mg; Iron, 281 mg; Zinc, 139 mg; Manganese, 64 mg; Copper, 24 mg; Cobalt, 0.08 mg; Iodine, 0.4 mg; Selenium, 0.3 mg; vitamin A, 12000 IU; vitamin D3, 1400 IU; vitamin E, 62 IU; Biotin, 0.6 mg; Riboflavin, 7 mg; Pantothenic acid, 25 mg; Niacin, 38 mg.

Table 3.2a Analyzed nutrient (g/kg) content of diets fed to non-pregnant sows

Nutrient	Barley-based diet		Corn-based diet	
	High protein	Low protein	High protein	Low protein
ME, MJ/kg	11.9	12.0	13.8	13.8
DM, g/kg	890.0	890.3	894.3	894.3
Crude protein, g/kg	13.6	11.1	12.1	9.2
Ether extracts, g/kg	27.2	13.9	42.4	22.9
Neutral detergent fibre, g/kg	159.5	155.2	134.3	126.6
Acid detergent fibre, g/kg	na	na	na	na
Ash, g/kg	73.8	67.7	71.6	65.4
Lysine[1], g/kg	4.6	4.6	4.6	4.6
Methionine + cysteine	5.1	4.0	4.0	3.3
Threonine	3.8	3.7	3.7	3.7
Tryptophan	1.2	0.9	1.0	0.9

[1]Grams of total amino acids per kilogram of diet.

Table 3.2b Analysed nutrient (g/kg) content of diets fed to gestating and lactating sows

Nutrient	Gestating diet		Lactating diet	
	High protein	Low protein	High protein	Low protein
ME, MJ/kg	14.7	13.8	15.2	14.6
DM, g/kg	898.0	894.3	907.7	899.7
Crude protein, g/kg	162.5	135.2	210.9	182.2
Ether extracts, g/kg	27.2	13.9	42.4	22.9
Neutral detergent fibre, g/kg	159.5	155.2	134.3	162.6
Ash, g/kg	73.8	67.7	71.6	65.4
Arginine[1]	8.7	6.2	12.4	8.6
Cysteine	3.4	2.9	3.9	3.6
Histidine	3.8	2.8	5.1	4.9
Isoleucine	5.6	4.3	8.0	5.6
Leucine	11.2	8.6	15.0	13.1
Lysine	6.6	6.8	9.3	9.2
Methionine	2.7	2.1	3.1	2.7
Phenylalanine	8.0	6.6	10.7	9.1
Threonine	5.6	5.2	7.0	6.5
Tryptophan	2.0	1.5	2.5	2.2
Valine	7.3	5.9	9.3	8.0

[1]Grams of total amino acids per kilogram of diet.

Table 3.3a Feed, protein and carbon intake (g/d) of non-pregnant sows[1] fed high or low protein diets based on barley or corn

Parameter	Barley based diet		Corn based diet			Effect, *P* =	
	HP[2]	LP[3]	HP	LP	SEM	Diet	Protein
Feed intake, g/d	2479[b]	2530[a]	2183[c]	2199[c]	37	0.58	0.001
Protein intake[4], g/d	362[a]	300[b]	289[c]	214[d]	10	0.001	0.001
Carbon intake, g/d	1093[a]	1099[a]	947[c]	956[b]	16.8	0.71	0.001

[1]No. of sows per treatment, 4; Mean body weight of sow, 237.8 ± 2.5 kg.

[2]High protein diet.

[3]Low protein amino acid supplemented diet.

[4]Calculated as N x 6.25.

Table 3.3b Feed intake (kg/d), protein and carbon intake (g/d) of high or low protein diets during two consecutive parities[1]

Parameter	Diet		Parity			Significance, P =	
	HP	LP	2	3	SEM	Diet	Parity
Gestation							
Feed intake, kg/d	2.38^{a}	2.34^{a}	2.25^{c}	2.46^{b}	0.04	NS	0.001
Protein intake[2], g/d	385.9^{a}	315.9^{b}	335.1^{d}	366.7^{c}	5.45	0.001	0.001
Carbon intake, g/d	1038.6^{a}	980.1^{b}	961^{d}	1057.8^{c}	32.2	0.001	0.001
Lactation							
Feed intake, kg/d	6.25^{a}	5.64^{b}	5.6^{d}	6.28^{c}	0.15	0.005	0.002
Protein intake[2], g/d	1317.3^{a}	1026.9^{b}	1106.9^{d}	1237.4^{c}	28.86	0.001	0.003
Carbon intake, g/d	2912.7^{a}	2504.4^{b}	2543.9^{d}	2873.2^{c}	63.1	0.001	0.005

[1]Values shown are least square means.

[2]Calculated as N x 6.25.

Table 3.4 Carbon dioxide and methane emission and carbon dioxide equivalents (g/d) produced by non-pregnant sows[1] fed diets at two protein levels based on barley or corn

Parameter	Barley based diet		Corn based diet		Significance, *P* =		
	HP[2]	LP[3]	HP	LP	Diet	Protein	D*P[4]
CO_2, g/d[5]	2912[b]	3090[a]	2761[d]	2906[c]	0.26	0.08	ns
SE	61	32	58	32			
Relative	100	106	100	105			
CH_4, g/d[6]	34.4[a]	14.8[b]	14.1[d]	18.8[c]	0.03	0.06	0.001
SE	2.2	3.1	2.2	2.2			
Relative	100	43	100	133			
CO_2-eq, g/d[6]	4885	3970	3592	3976	0.16	0.002	ns
SE	165	233	165	165			
Relative[7]	100	81	100	111			

[1]No. of sows per treatment, 4; Mean body weight of sow, 237.8 ± 2.5 kg.

[2]High protein diet.

[3]Low protein amino acid supplemented diet.

[4]Diet and protein interaction.

[5]Means adjusted for feed intake.

[6]Least square means.

[7]Relative to HP (100) within type of ingredient.

Table 3.5 Carbon dioxide and methane emission (g/d) from non-pregnant sows[1] fed high or low protein diets based on barley or corn

Greenhouse gas	Barley based diet		Corn based diet			Significance, P =	
	HP[2]	LP[3]	HP	LP	SEM	Diet	Protein
CO_2, g/d[4]	2894	3109	2779	2888	46	0.07	0.06
CH_4, g/d[5]	34.5[a6]	14.8[b]	14.1[d]	18.8[c]	2	0.006	0.003
CO_2-eq, g/d[5]	4885[a]	3970[b]	3591[d]	3976[c]	128	0.16	0.002

[1]No. of sows per treatment, 4; Mean body weight of sow, 237.8 ± 2.5 kg.

[2]High protein diet.

[3]Low protein amino acid supplemented diet.

[4]Effect of feed intake, $P < 0.01$.

[5]Effect of protein level * diet interaction, $P < 0.001$.

[6]Different letters in a row denote differences, $P < 0.05$.

Table 3.6 Carbon dioxide equivalents (g/d) per non-pregnant sow[1] fed high or low protein diets based on barley or corn

CO_2-eq[2]	Barley based diet		Corn based diet			Significance, P =		
	HP[3]	LP[4]	HP	LP	SEM	Diet	Protein	D*P[5]
$CO_2 + CH_4$	4885[a5]	3970[b]	3592[b]	3976[b]	130	0.002	0.16	0.001
N excretion	28216[a]	23348[b]	22530[b]	16610[c]	782	0.001	0.001	0.002
Estimated[7]	13350[a]	10974[b]	10351[b]	8959[c]	354	0.001	0.001	0.001

[1]Mean body weight of sow, 237.8 ± 2.5 kg.

[2]Carbon dioxide equivalents.

[3]High protein diet.

[4]Low protein amino acid supplemented diet.

[5]Effect of protein level * diet interaction, $P < 0.001$.

[6]Different letters in a row denote differences, $P < 0.05$.

[7]Estimated greenhouse gas emissions from sow (i.e., $CO_2 + CH_4$), assuming sow is at N equilibrium, and 30 % N excreted is converted to N_2O. Applied the global warming potential factors of 1, 23, and 310 for CO_2, CH_4 and N_2O (IPCC, 1997), respectively.

Table 3.7a Carbon and nitrogen excretion (g/d) during gestation and lactation in sows[1] fed high or low protein diets based on barley or corn

Nutrient	Gestation diet		Lactation diet			Significance, P =	
	HP	LP	HP	LP	SEM	Protein	Phys.[2]
Fecal C, g/d	193[a]	187[a]	523[b]	488[c]	13.1	0.08	0.001
CO_2, g/d	2691[a]	2571[b]	5930[c]	5550[d]	123	0.002	0.001
Fecal N, g/d	68.3[a]	64.2[a]	242.0[b]	236.7[b]	7.0	0.38	0.001
N:Cr[3]	5.2[a]	4.2[b]	11.1[c]	7.8[d]	0.3	0.001	0.001

[1]No. of sows per physiological state, 22.

[2]Physiological state of sows.

[3]N:Creatinine ratio; protein level * physiological state, $P = 0.008$.

Table 3.7b Effect of diet, stage and parity on CO_2 emission (g/d) from sows during gestation and lactation[1]

Parameter			SEM	Significance, P =
Gestation				
Diet	HP	LP		
	3024.2	2861.2	44.5	0.01
Stage	Early	Late		
	2860.2	3025.2	697.6	0.009
Parity	2	3		
	2774.0	3111.4	0.03	0.007
Lactation (sow without litter)				
Diet	HP	LP		
	4535.0	4359.6	101.6	NS
Stage	Early	Late		
	4533.8	4360.8	101.6	NS
Parity	2	3		
	4194.9	4702.7	101.6	0.001
Lactation (sow and litter)				
Diet	HP	LP		
	6482.1	6315.5	69.8	0.1
Stage	Early	Late		
	6113.3	6684.3	69.8	0.001
Parity	2	3		
	6257.1	6540.5	69.8	0.007

[1]Values are least square means adjusted for feed intake as covariate.

Table 3.8 Carbon dioxide equivalents (g/d) during gestation and lactation in sows[1] fed high or low protein diets based on barley or corn

CO_2-eq[2]	Gestation diet		Lactation diet			Significance, P =	
	HP[3]	LP[4]	HP	LP	SEM	Protein	Gest. vs. Lact.[5]
CO_2-eq	2691	2571	5930	5550	123	0.002	0.001
CO_2-eq: C[6]	14847	14432	40253	37606	1006	0.08	0.001
CO_2-eq: N[7]	33248	31282	117874	115313	3401	0.39	0.001
CO_2-eq[8]	17120	16285	53368	51425	1439	0.21	0.001

[1]No. of sows per physiological state, 22.

[2]Carbon dioxide equivalents

[3]High protein diet.

[4]Low protein amino acid supplemented diet.

[5]Gestation versus lactation

[6]Potential carbon dioxide equivalents per sow based on fecal C excretion only, assuming 20% of excreted C is converted to CO_2.

[7]Potential carbon dioxide equivalents per sow based on fecal N excretion only, assuming 30% of excreted N is converted to N_2O.

[8]Carbon dioxide equivalents, excluding emissions from urinary C and N excretion, but from combined fecal C and N, assuming 3% of C and 6% of N converted to CO_2 and N_2O respectively.

Table 3.9 CO_2-equivalents produced by one sow per year when fed conventional or reduced protein diet

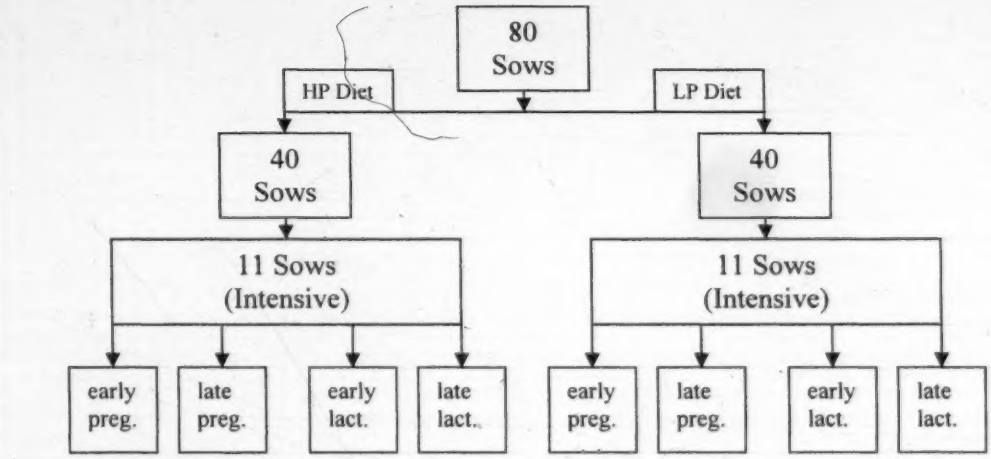
Parameter	Conventional diet		Reduced protein diet	
	Gestation	Lactation	Gestation	Lactation
CH_4, g/d[1]	26.7	76.8	16.9	45.8
CO_2-eq[2] from CH_4, g/d	1541.9	4435.2	976.0	2645.0
CO_2-eq from CO_2, g/d	3024.2	6482.1	2861.2	6315.5
CO_2-eq (total), g/d	4566.1	10917.3	3837.2	8960.5
CO_2-eq, kg/yr[3]	2015.9		1682.4	
Relative[4], %	100.0		83.4	

[1]Metabolizable energy intake, 0.821 g/MJ and 0.558 g/MJ for conventional and reduced protein diets respectively.

[2]Carbon dioxide equivalents.

[3]115 d gestation, plus 16 d non-pregnant, 23 d lactation, 2.37 reproductive cycles per sow in year.

[4]HP diet taken as 100.



early preg. = early pregnancy, late preg. = late pregnancy; early lact. = early lactation, late lact. = late lactation

Figure 3.1 Schematic representation of experimental design.

4.0 EFFECT OF PROTEIN AND ENERGY SOURCE AND PROTEIN INTAKE ON PERFORMANCE, NUTRIENT EXCRETION AND GREENHOUSE GAS EMISSIONS BY GROWING-FINISHING PIGS

4.1 Introduction

Increased public concerns and regulatory pressures that the pig industry is depositing excessive nitrogen into the environment, potentially contaminating soil and water supplies have necessitated the re-examination of traditional diet formulation and feeding practices. Experimental evidence indicates that the environmental impact of pork production can be reduced through diet modification (Ferket et al., 2002; Lenis, 1989; Gatel and Grosjean, 1992; Jongbloed and Lenis, 1992). One such method is to reduce the crude protein content of the diet by providing limiting amino acids in synthetic form. Reducing dietary crude protein while supplementing free amino acids usually lowers N excretion (Fremaut and Deschrijver, 1991; Möhn and Susenbeth, 1995; Quiniou et al., 1995; Hobbs et al., 1996; Sutton et al., 1996; Canh et al., 1998; Misselbrook et al., 1998; Lenis and Jongbloed, 1999; Zervas and Zijlstra, 2000a, b), and maintains pig performance (Bourdon et al., 1995; Le Bellego et al., 2001; Ball and Moehn, 2003). The efficacy of low protein amino acid supplemented diets in reducing nitrogen excretion by growing pigs has been demonstrated (Jongbloed and Lenis, 1992; Kerr and Easter, 1995; Lee et al., 2001). However, reduced performance of finisher pigs has also been observed (Kendall et al., 2000; Schoenherr, 1992; Tuitoek et al., 1997a, b). Protein reduction is achieved by replacing protein-bound ingredients like soybean meal with appropriate amounts of limiting amino acids such as lysine, methionine, threonine and tryptophan, the lack of which would otherwise limit performance (Gatel and Grosjean, 1992; Lee et al., 1993; Lenis and Jongbloed, 1999; Roth and Kirchgessner, 1993).

Reduced protein diets have been associated with a reduction in energy losses. Reducing dietary protein concentration lowers deamination of excess amino acids and the subsequent synthesis and excretion of urea in urine and lowers body protein turnover and heat energy (i.e., heat production) of the animals (Fuller et al., 1987; Noblet et al., 1987; Roth et al., 1999). Thus, reducing dietary protein concentration increases the energy

available for tissue deposition. This agrees with the net energy system (Noblet, et al., 1994), which assumes that proteins are less efficiently used than starch (i.e. carbohydrate), 60 vs. 80% for metabolizable energy. However, the net energy system was established with higher dietary crude protein levels (around 17.3% on average) than those currently used for growing-finishing pigs.

Reducing dietary crude protein concentration increases dietary carbohydrates content because protein-bound feeds, such as soybean or canola meal, are replaced with cereal grains containing a large amount of starch. Starch is more efficiently used for fat deposition than amino acids (84% vs. 52%; van Milgen et al., 2001), and starch contains 40 % carbon, while amino acids contain on average 52 % carbon (Schiemann et al., 1971). Carbohydrates also have lower carbon content and are used more efficiently in intermediary energy metabolism (Roth and Kirchgessner, 1993) than protein. Reducing dietary crude protein content will therefore reduce carbon content and increase the efficiency of carbon utilization, from which a reduction in CO_2 production by animals and a reduction in carbon in the excreted manure can both be expected.

Reducing dietary crude protein also changes the content of dietary fibre (fermentable fibre), and has the potential to lower the CH_4 production by pigs because CH_4 emissions appear linearly related to the intake of non-starch polysaccharides (NSP) (Jensen, 1996). Therefore, the cereal on which swine diets in western Canada are predominantly based (barley) may affect CH_4 emissions. To the best of the authors' knowledge, the effects of these dietary changes on CH_4 emission by pigs have not been quantified. Based upon these concepts, low-protein, amino acid-supplemented diets were hypothesized to reduce the emission of CO_2, CH_4 and heat production by growing-finishing without sacrificing animal performance. The objectives of this study were to assess the effect of high- and low-protein diets based on corn-soybean meal (CS) or wheat- barley-canola meal (WBC) on performance, N excretion, CO_2, and CH_4 emissions from growing-finishing pigs.

4.2 Materials and Methods

The experiment was approved by the Faculty Animal Policy and Welfare Committee for adherence to the Canadian Council of Animal Care (CCAC, 1993) guidelines.

4.2.1 Experimental Design

Twenty-four female growing-finishing pigs of an average initial body weight of 50 ± 0.8 kg were randomly allocated to four dietary treatments in a cross-over design (Figure 4.1) based on either WBC or CS. Within each ingredient group, twelve pigs were offered either conventional, high-protein (HP) or reduced-protein, amino acid supplemented (LP) diets. Each pig was an experimental unit and served as its own control. The pigs were adapted to diet and housing for at least 7 d. During the adaptation period, the pigs were repeatedly confined in respiration chambers to acclimatize them to the respiration boxes (measuring 234.5 cm x 106 cm x 86 cm per chamber) prior to start of respiration studies. After additional 3 d adaptation to adjustable metabolism crates with rubber-coated mesh floor (measuring 183 cm x 61 cm x 88 cm; and 62 cm above concrete floor), 7 d nitrogen balance with quantitative collection of feces and urine was performed (Figure 4.2). Feces voided were collected frequently and stored in covered plastic buckets. Buckets with collected feces were stored in cooler at -4°C overnight at 1800 h at the end of each collection day till the following morning at 0600 h.

During the balance period, early morning fecal collections on each day were made at 0600 h. At the end of a balance period, the pooled 7 d fecal collection per animal within diet was weighed, thawed and homogenized. Sub-samples (360 ± 10 g) of homogenized feces were placed in aluminum pans (in duplicate) covered with polyethylene bags and stored at -20°C prior to freeze drying. Freeze dried samples were ground with a coffee grinder and stored in sealed plastic containers until chemical analyses. Daily urine collections were done over 20 ml concentrated H_2SO_4 to prevent volatilization of urine N as NH_3 and prevent bacterial growth, in plastic buckets with lids placed under the V-shaped stainless steel collection tray covered with 0.5 mm screen preventing fecal materials from contaminating the urine collections. The 24 h daily urine collections were from 1000 h to 1000 h the following day on each day during the balance period. At the

end of each 24 h collection, the urine was weighed, thoroughly stirred and sub-sampled. Five percent aliquot samples (w/w) from daily collections were pooled into brown bottles stored in cooler till end of balance collections per animal within diet. At the end of the 7 d the pooled aliquots of urine were weighed, thoroughly homogenized, and sub-sampled. Sub-sampled urine collections (100 ml) per animal within diet were stored at -20°C in plastic containers till thawed for chemical analyses. Immediately after the nitrogen balance study 4 h respiration studies were performed using open circuit indirect calorimetry. The pigs were then switched to another diet, and the nitrogen balance and respiration studies were repeated (Figures 4.1 and 4.2). In four of the pigs fed CS diets, the respiration measurements (O_2 consumption, CO_2 and CH_4 emission) were continued for 24 h to enable extrapolation of 4 h to 24 h respiration measurements. Animals were weighed weekly, and before and after balance periods.

4.2.2 Diet Composition

Four diets were formulated based on WBC or CS. For each type of ingredients, conventional diets (HP) and protein-reduced amino acid supplemented diets (LP) were formulated (Table 4.1). The reference diet was the gilt developer diet at the Swine Research and Technology Center, University of Alberta (Aherne and Foxcroft, 2000). The WBC-HP and WBC-LP were formulated to achieve equal metabolizable energy (ME) content and equal true ileal digestible contents of lysine, methionine, threonine and tryptophan. The CS-HP and CS-LP diets were formulated to achieve a lysine to ME ratio similar to that of the WBC diets. Vitamin and mineral contents of all diets met or exceeded the recommendations of NRC (1998). The nutrient contents of the diets are shown in Table 4.2.

4.2.3 Housing and Feeding

The growing-finishing female pigs were received from the closed population of group-housed pigs at the Swine Research and Technology Centre and individually housed at the Metabolic Unit, University of Alberta research station in four batches of six (Figure 4.2). The animals were adapted to individual housing and diet for 7 to 10 days prior to the nitrogen balance study (Figures 4.1 and 4.2). The WBC diets were fed restrictively at a

rate of 90% of ad libitum feed intake according to NRC (1998). The CS diets were offered at a lower rate to achieve equal energy intake to the WBC diets. Feed not eaten was collected, dried, weighed and deducted from the daily feed allowance to calculate net feed intake. Feed was offered twice daily, except for the respiration studies during which half the daily allowance was offered in eight equal hourly meals, two hours prior to the commencement of respiration studies to facilitate feed consumption and reduce wastage in the chambers. This also made it possible to monitor the activities (feeding and resting and gut motility patterns) and relate these to the respiration measurements. Water was supplied ad libitum from a low pressure nipple.

4.2.4 Growth Measurements

Body weights of the pigs were measured weekly, and before and after nitrogen balance and respiration studies on a scale (Accurate Scale Industries, Ltd., Edmonton, AB) with a digital readout (DF 2000, Massload Technologies, Saskatoon, SK).

4.2.5 Respiration Measurements

The respiration chambers (234.5 cm x 86 cm x 106 cm per chamber) consisted of commercial farrowing crates enclosed in plexiglass boxes, equipped with a feeder and low pressure nipple drinker, and a cooler to maintain temperature in the thermoneutral zone. Air was drawn through boxes via an inlet at rear and an outlet above the feed trough at rates of approximately 240 to 250 L/min. Air flow was measured after passing drawn air through a cold water condenser, to remove water vapor, with temperature-compensated commercial air meters (Model 1023, Canadian Meter Corp., Cambridge, Canada). A sample of air was withdrawn with small air pump (Gast Model 0531, Gast Mfg. Corp., Benton Harbour, MI) and delivered to a fuel-cell type O_2 (Taylor-Servomex, Crowborough, UK) and a non-dispersive near infra-red CO_2 analyzer (Beckman LB2, Beckman, Irvine, CA), and CH_4 analyzers (Qubit Systems, Kingston, ON, Canada). Air flow to the analyzers was regulated to 0.5 L/min by ball-type flow meters (Scienceware Size 2, Fisher Scientific, Mississauga, Canada). The analog output (mV) of the analyzers was converted to digital data by an analog-digital converter (Data grabber, Data Electronics, Australia) and recorded by a computer. Data acquisition was set for

maximum rate (four readings per second) and the average gas concentration for each minute was recorded. For each study, the O_2 and CO_2 and CH_4 (Qubit Systems, Kingston, ON, Canada) analyzers were calibrated for zero and gain readings with either pure N_2 (zero) or calibration gas (1% CO_2, 20% O_2, and 79% N_2). The span gas was composed of 19.44% O_2, 1.53% CO_2, 105 ppm CH_4 and balance N_2. Measurements of these gases at steady state and room air were recorded before and after the study period. Measurements were continuously recorded for four hours and animals were fed one quarter of their daily ration every hour. During the entire 5 h respiration measurements (1 h equilibration plus 4 h study) the pigs were confined in the respiration chamber. Expired air was analyzed continuously for O_2, CO_2 and CH_4 contents during the 4 h. A similar procedure was repeated for the four pigs that underwent 24 h respiration. However, during the 24 h the pigs received half of their daily allowance in eight hourly meals.

4.2.6 Chemical Analyses

Feed, fecal and urine samples collected were stored at -20°C prior to chemical analyses. Feed samples from the experiment were taken weekly, and pooled within diet type. Pooled feed samples were analyzed for amino acids by Degussa AG, (Hanau, Germany) using ion exchange chromatography (Llames and Fontaine, 1994). Tryptophan was analyzed by fluorescence detection HPLC after alkaline hydrolysis with barium hydroxide octahydrate at 110°C for 20 h (Fontaine et al., 1998). Gross energy of feed and fecal samples was determined using an adiabatic bomb calorimeter (Leco AC-300, LECO Corp., St. Joseph, MI). Feed, fecal and urine N content were determined according to micro-Kjeldahl (AOAC, 1998). Proximate analyses involving dry matter, crude protein (macro-Kjeldahl, AOAC, 1998), ash, ether extract (Goldfisch extraction apparatus, Labconco Corp., Kansas City, MO), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Ankom Fiber Technique, Goering and van Soest, 1970) were determined on feed and fecal samples. Total carbon content of feed and freeze-dried fecal samples ground in a MM2 Retsch/Birkmann mixer mill (Brinkmann Instruments Inc., Westbury, NY), were analyzed using a NA 1500 Carlo-Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ). Prior to proximate

analyses, feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a 1.0 mm screen

4.2.7 Calculations

During the nitrogen balance period, whole tract digestibilities of nitrogen, phosphorus, calcium, ether extracts, NDF, ADF and gross energy were determined. Lipid and protein energy retention were calculated according to C-N balance technique, assuming 23.8 kJ/g deposited protein, and 39.6 kJ/g deposited lipid (Noblet et al., 1987; Moehn and Susenbeth, 1995). The amount of digested nutrients and energy, and nitrogen and energy excretion in urine, was measured. Digested protein (DP) and fat (DF) and carbohydrate (DCHO) expressed in energy units were calculated from the 7 d balance experiments as:

DP, kJ = diN x 6.25 x 23.86 kJ/g, where diN (g) = digested nitrogen,

DF, kJ = diEE x 39.76 kJ/g, where diEE (g) = digested fat

DCHO, kJ = DE, kJ – DP, kJ – DF, kJ, where, DE = digestible energy.

Maintenance net energy (NEm) was taken as the intercept of a regression of heat production (HE) on feed intake (per $kg^{0.60}$ body weight, Noblet et al., 1987). Dietary net energy (NE) was calculated as retained energy (RE) plus maintenance net energy (NEm). After determination of NDF, ADF and ADL values for feed and fecal samples, using the modified method of Goering and van Soest (1970), hemicellulose was calculated as difference between NDF and ADF. Cellulose content of feed and feces was calculated as difference between ADF and ADL.

The set of factors and constants from Brouwer, (1965) were used in all calculations. HE based on the measurements of gas exchange and 7 d determination of nitrogen excreted in urine (U_N) was calculated as:

$HE\ (kJ) = 16.18 * V_{O2} + 5.02 * V_{CO2} - 5.99 * U_N - 2.17 * V_{CH4}$ (Brouwer, 1965).

HE is heat production, V is the volume of gas in litres, and U_N is nitrogen excreted into urine in grams.

Retained (or mobilized) energy in protein (RP) was calculated from the nitrogen balances and gas exchange measurements as:

RP, kJ = (diN – U_N) x 6.25 x 23.86 kJ/g

Retained (or mobilized) energy in fat (RF) was calculated either as:

RF, kJ = ME, kJ – HE, kJ – RP, kJ, where ME = metabolizable energy (kJ); HE = heat production (kJ); RP = retained energy in protein (kJ). The RP and RF were also calculated from carbon (C) balances as:

C in CO_2 emitted, g = CO_2 production, litres x 0.536, g/L

C in RP, g = RP, g x 0.52, where 0.52 is average C content in protein

C balance, g = C intake, g – (C in feces, g + C in urine, g + C in CO_2, g)

C in RP, g = C balance, g – C in RP, g

RF, kJ = C in RF, g/0.767 x 39.76, kJ/g, where 0.767 is an average C content in fat.

Carbon (C) retention was calculated as:

$C_{intake} - C_{in\ feces} - C_{in\ urine} - C_{in\ CO2} - C_{in\ CH4}$.

It was assumed that the carbohydrate content of the animals remained constant during the respiration period. The C content was taken as: 12 g/mol for CO_2 and CH_4, and as 90% of the N content of urine (Schiemann et al., 1971). Lipid retention was calculated as: $C_{retention}$ minus $C_{retained}$ in protein (52%) divided by the C content in body fat (76.7%), as reported by Schiemann et al. (1971).

Metabolizable energy (ME) intake was calculated as:

ME, kJ = DE – CH_4E – UE, where DE = digestible energy (kJ/g); CH_4E = energy in CH_4 (39.56 kJ/L; Schiemann et al., 1971); UE = energy in urine (UE, kJ/g = 0.333 * % C + 0.093 * % N, Hoffmann and Klein, 1980). RE taken as energy retained as protein and fat (22.6 kJ/g and 39.6 kJ/g respectively; Böhme and Gädecken, 1980).

Respiratory quotient (RQ), based on the measurements of gas exchange and 7 d determination of nitrogen excreted in urine (UN) was calculated as:

RQ = (V_{CO2} – [U_N x 6.25 x 0.774])/(V_{O2} – [U_N x 6.25 x 0.957]), where, V_{CO2} = volume of CO_2 emission (litres); V_{O2} = volume of O_2 consumption (litres); UN = urinary nitrogen (g). In its simplistic form the equation for RQ is expressed as: RQ = V_{CO2}/ V_{O2}.

The RQs were calculated to determine the types of substrate utilized by the growing-finishing pigs during the study period.

CO_2-equivalents (CO_2-eq), from the growing-finishing pigs, were calculated using factors of the global warming potential (GWP): 1, 21 and 310 for CO_2, CH_4 and N_2O respectively, on a molar basis (Grubb et al., 1999; IPCC, 1997). The equations were as follows:

CO_2-eq (CO_2) = CO_2, d, where CO_2, d = CO_2, g x 1440/1.114.

CO_2-eq (CH_4) = 21 x 44/16 x CH_4, d, where CH_4, d = CH_4, g x 1440/0.627.

CO_2-eq from pigs (CH_4+ CO_2) = 21 x 44/16 x CH_4, d + CO_2, d.

The conversion of nutrients in manure to greenhouse gase, is not well established, therefore, the range in CO_2-equivalents from manure were calculated using estimated conversion rates of 5 or 30% of the N in manure to N_2O (Béline et al., 1999), and 3 or 20% of C in manure to CH_4 (Martinez et al., 1999). The contents of N and C in manure were taken as the excreted N and C by the pigs. Total excretion values (TN and TC respectively) per pig were calculated as the sum of fecal and urinary N and C excretion. Lipid and energy retention were calculated using the C-N balance technique.

4.2.8 Statistical Analyses

The effects of type of diet and protein level were estimated using the Proc mixed model procedures (SAS, 2005). The interaction between type of diet and protein level was tested for all outcome parameters and retained in the model if $P < 0.10$. Least squares means (LSM) were calculated and the probability of differences between treatments determined with the 'pdiff' option, which performs a pair-wise comparison based on a two-tailed t-test. Significance was set as $P < 0.05$, and all P-values between 0.05 and 0.1 were regarded as trends.

4.3 Results

The chemical composition of the diets (Table 4.2) is in agreement with the objectives of the experiment.

4.3.1 Animal Performance

Data given in Table 4.3 indicate that, during the 7-d collection period final body weight, body weight gain, average daily feed intake and intake of metabolizable energy were not affected ($P > 0.1$) by protein level in both WBC and CS diets.

The lower feed allowance for the CS diets was found to be insufficient to offset the greater metabolizable energy content of the diet. Therefore, the metabolizable energy intake was slightly greater ($P = 0.01$) for CS than WBC based diets.

Nitrogen Balance

Results of the N balance indicate a clear reduction ($P < 0.01$) in N intake (Table 4.3) and total N excretion (Table 4.4) due to protein effects. Urinary N excretion was reduced ($P < 0.01$, Table 4.4). These reductions in N excretion were achieved by affecting the quantity of N retained by the animals resulting from a protein ($P = 0.001$) and not diet ($P = 0.21$) effect. Interactions between diet ingredient type and protein level were not significant ($P > 0.1$) except for daily nitrogen retention. The N retention of pigs fed the CS diets was affected ($P = 0.001$) by protein level due to the lower than expected N retention in pigs fed the CS-LP (Table 4.3). Neither protein level nor type of ingredients affected ($P > 0.10$) energy retention. Heat production was not different between protein levels ($P = 0.18$) or type of ingredients ($P = 0.75$).

4.3.2 Nutrient Excretion (N and C)

The fecal excretion of nitrogen did not differ ($P = 0.42$) between ingredient types, but there was a significant protein effect ($P = 0.05$) with fecal N lower in LP diets than HP (Table 4-4). The urinary N excretion was lower for the WBC than CS diets, and lower for the low-protein treatments, with significant diet ($P = 0.001$) and protein ($P = 0.002$) effects. The magnitude of urinary N excretion (g/d) was greater than for fecal N. The total N excretion was reduced by lowering the dietary protein contents, and was lower for

WBC than CS diets (Table 4.4). The total N excretion was reduced by 23.7% for the WBC-LP diet, and 8.3% for the CS-LP diet compared to the WBC-HP diet and CS-HP diet, respectively. The mean reduction in N excretion by reducing dietary protein was 16%. The fecal carbon excretion was not different ($P = 0.98$) between protein levels, but was lower for CS than for WBC diets ($P = 0.001$). The total C excretion was not affected ($P = 0.36$) by protein level, and was lower for the CS compared to the WBC diets (Table 4.4).

4.3.3 Oxygen Consumption, CO_2 and CH_4 Emission

The linear extrapolation of 4 to 24 h oxygen consumption, CO_2 and CH_4 emissions (Table 4.5) were based on respiration data from four of the twelve pigs fed the CS diets. The CO_2 emission was higher (11%) for 4 h measured values than estimated 24 h values. Similarly, oxygen consumption was 11% higher in 4h than estimated 24 h values. Respiration quotient values of 1.14 and 1.11, respectively. On the contrary, estimated 24 h value for CH_4 emission was higher than measured 4 h emission (33.6 ± 2.4 vs. 23.3 ± 3).

4.3.4 Gas Exchange and GHG Emission

The CO_2 production was lower ($P = 0.04$) for CS than WBC diets, and not different ($P = 0.38$) for the LP than HP diets (Table 4.6). The CH_4 production was lower ($P = 0.05$) for LP than HP diets, but not different ($P = 0.12$) between CS and WBC diets (Table 4.6). The CH_4 production was correlated ($P < 0.05$) to the dietary NDF (Table 4.2) and hemicellulose content (data not shown), which declined with reduced protein content.

The average gaseous excretion of CO_2-equivalents of 3,181 g/d (CO_2 and CH_4 emission by the pigs) was not different between diet type ($P = 0.98$), but tended to be lower ($P = 0.09$) for LP than HP diets (Table 4.6). The reduction in CO_2-equivalents emitted by the pigs was 14.3% for WBC-LP and 7.4% for CS-LP diets compared to the WBC-HP and CS-HP diets, respectively.

4.4 Discussion

The objectives of this study were to assess the effect of high- and low-protein diets based on corn-soybean meal (CS) or wheat- barley-canola meal (WBC) on performance, N excretion, CO_2, and CH_4 emissions from growing-finishing pigs. The dietary CP contents were reduced by 3.5 and 3.6 percentage units (with supplementation of L-lysine HCl, DL-methionine, L-threonine and L-tryptophan) in the WBC and CS diets, respectively.

Reducing dietary protein maintained performance and reduced nutrient excretion with WBC based diets. The performance of the growing-finishing pigs given nitrogen-reduced diets and balanced with adequate amounts of industrial amino acids is in agreement with published results (Spiekers et al., 1991; Valaja and Alavinhkola, 1993; Kay and Lee, 1996). Further, as was examined with these feeding regimens, nitrogen excretion per unit weight gain could be decreased by about 30 to 35% without negatively impacting gain and N retention in WBC (Table 4.3). These results are in accordance with findings described by in the literature (Kirchgessner and Roth, 1993; Bourdon et al., 1995; Bridges et al., 1995; Kay and Lee, 1996; Nonn et al., 1997; Canh et al., 1998). On the contrary, supplementing similar synthetic amino acids Kendall et al. (2000), Schoenherr (1992), and Tuitoek et al. (1997a, b) observed reduced performance of finisher pigs. In other studies reported in the literature, protein reduction was achieved by replacing protein-bound ingredients like soybean meal with appropriate amounts of limiting amino acids such as lysine, methionine, threonine or tryptophan, but observed limited performance (Gatel and Grosjean, 1992; Lee et al., 1993; Lenis and Jongbloed, 1999; Roth and Kirchgessner, 1993). In agreement with results of Dourmad et al. (1993), N excretion in growing-finishing pigs can be reduced by about 10% for each percentage-point CP reduction in the feed. The results also confirmed that excess protein intake is mainly excreted in the urine (Canh et al., 1998) as shown in Table 4.4. In agreement with studies of Le Bellego et al. (2001), Dourmad et al. (1993), Tuitoek et al. (1997) and Canh et al. (1998), our results demonstrate that reduction of CP level, while maintaining essential AA in the feed, does not affect the N retention by the animals in WBC, but not in CS where intake is reduced.

The reduction of retained nitrogen from the CS diet and (or) energy retention from the WBC with low-CP diets can be attributed to the balance technique, as previously demonstrated by Quiniou et al. (1995). Indeed, this technique underestimates N losses and consequently overestimates N retention, and the overestimation becomes more important as CP level in the diet or N losses are elevated. This observation also emphasizes the inadequacy of the balance technique for estimating N retention at variable CP intakes. This explanation is supported by growth performance of pigs in this study. Thus, when low-CP diets are balanced in terms of essential AA and adequate for ratios between energy and protein supply, it is feasible to reduce CP levels by 3 or 4 pu without affecting N retention, lean tissue growth or BW gain.

The fat retention tended to be greater ($P = 0.06$) for the LP than the HP diets, but was not significantly different between the CS and the WBC diets ($P = 0.16$) (Table 4.3). This meant that N retention was limited by intake of an amino acid from the LP diets. Thus energy was retained as fat rather than used to synthesize more protein. Our results are in agreement with findings of Quiniou et al. (1995), in a study comparing two measurement methods to evaluate effect dietary CP level on protein and energy balance. LP pigs retained more energy as fat, and less energy as protein than HP pigs.

LP diets have been associated with a reduction in energy losses (Fuller et al., 1987; Noblet et al., 1987; Roth et al., 1999). Therefore, reducing CP increases the energy available for tissue deposition. Our results showed reduced heat production in LP compared to HP diets within the ingredient types, but failed to reach significance (Table 4.3). Methane energy losses with reference to CH_4 emission are expected to follow similar pattern to heat production, with significant protein ($P = 0.05$), and not diet ($P = 0.12$) effect (Table 4.6).

Over all, reducing the dietary protein reduced N excretion and the CH_4 emission by pigs, but led to only a marginal reduction in CO_2 production. The CO_2-equivalents arising from the pigs themselves – from CO_2 and CH_4 production – tended to be lower with reduced dietary protein. This shows that LP diets increase the efficiency of energy (carbon) and N

(protein) retention. Further GHG emission will depend on the conversion of the carbon and N excreted into manure into CO_2, CH_4, and N_2O. Our results show that reducing dietary protein intake reduces the N excretion by pig and has the potential to reduce GHG emissions by pigs by over 20%. To realize the full potential, the conversion of nutrients in manure into GHG must be investigated further, along with means to influence this conversion.

Energy metabolism and substrate oxidation were measured by means of indirect calorimetry and nutrient balance. The RQ value of 1.14 (4 h) and 1.11 (based on 24 h extrapolated values) indicate that during the feeding period, dietary carbohydrates were the main energy source, sufficient to cover energy requirement without oxidation of fat (Table 4.5). Such RQ values for 4 h compare favourably with values obtained during 24 h respiration studies (Chwalibog et al., 2004). Under normal feeding conditions, the main source of energy for growing-finishing pigs is dietary carbohydrates followed by protein, and provided there is enough energy from carbohydrate and protein to sustain requirements for maintenance and growth, dietary fat is not oxidized but retained in the body (Chwalibog and Thorbek, 2000). However, during post-absorption mobilized body fat becomes the main source of energy followed by body protein oxidation (Chwalibog et al., 2004). This situation is changed during re-feeding as macronutrients are again available from the diet. Consequently, the metabolism will change from utilizing body fat and protein reserves to using dietary nutrients.

The switch between endogenous and exogenous substrates is not an immediate process and a certain time is necessary to re-establish metabolism from catabolic to anabolic conditions. It is also assumed that our calculations do not describe individual pathways of nutrient metabolism but the general relations between substrates and product. The calculations were carried out in accordance with the method described in Section 4.2, using constants and factors generally accepted for studies in energy metabolism in animals (Brouwer, 1965; Wenk et al., 2001).

Our results are based on measurement of oxygen consumption and CO_2 emission by indirect calorimetry and urinary nitrogen excretion. Calculations of energy expenditure and substrate oxidation are sensitive to the accuracy of measurements of gas exchange (Jebb et al., 1996) and can be biased by the potential pitfalls in the interpretation of data from gas exchange measurements (Elia and Livesey, 1988; Ferrannini, 1988; Chwalibog and Thorbek, 2000). However, the gas exchange measurements was high in our experiment with an overall standard error of less than ±1.5% compared to the standards. In addition, errors in collection of urine and unaccounted for evaporation of ammonia may cause an underestimation of the urinary nitrogen and consequently heat production may be underestimated.

The difference in excreted C, between HP and LP fed pigs, has implications for GHG production from manure; if quantitatively converted to CH_4, it would amount to 7,821 g/d of CO_2-equivalents. Such a reduction is attributable to the effect of diet manipulation on the pigs themselves and does take into account any additional effects on GHG emissions related to manure that will contain less N and fermentable C. The CH_4 resulting from pig production also depends on the transformation of excreted N into N_2O (Béline et al., 1999). If quantitatively transformed, 19,400 g/d CO_2-equivalents could be produced. However, when aerating manure 5 to 30% of the N in manure may be lost as N_2O (Béline et al., 1999). Assuming these rates of N_2O loss leads to a two-fold difference in CO_2-equivalents of 3,919 (SE 107) g/d vs 8,365 (SE 213) g/d, it will affect data interpretation: the greater rate shows a significant diet effect, but the lower does not. However, reducing dietary protein contents reduced the production of CO_2-equivalents even at the lower rate of conversion.

4.5 Conclusion

Lowering dietary protein maintained animal performance, reduced GHG emissions by pig, reduced N and C excretion, and shows potential for reduced GHG emissions from excreta.

4.6 Implications

Dietary manipulation can reduce greenhouse gas production by pigs. GHG production by growing-finishing pigs can be reduced by at least 25% by feeding amino acid supplemented low-protein diets. This was accomplished by decreasing CO_2 (3.8%), CH_4 (27.3%) and nitrogen excretion (24%). The CO_2-equivalents were reduced regardless of the degree of conversion of nutrients in manure. Reducing dietary protein is a tool to reduce GHG emissions independent of handling or technological manipulation of manure. Diet manipulation should be used to reduce GHG emissions prior to additional investment into expensive manure management technologies.

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Table 4.1 Ingredient composition (g/kg) of experimental diets

Ingredient	Wheat-barley-canola based diets		Corn-soybean meal based diets	
	High protein	Low protein	High protein	Low protein
Wheat	290.0	246.0	0	0
Barley	500.0	665.8	0	0
Corn	0	0	754.2	841.3
Tallow	10.0	10.0	5.8	0
Soybean meal	100.0	49.0	215.0	131.0
Canola meal	75.0	0	0	0
L-lysine HCl	0	2.7	0	2.4
DL-methionine	0	0.1	0	0.3
L-threonine	0	1.3	0	0.7
L-tryptophan	0	0.1	0	0.3
Vitamin-mineral premix[a]	25.0	25.0	25.0	25.0

[a]Provided per kilogram of premix: Ca, 21.5%; available P, 8.4%; Na, 4.8%; Mg, 1%; Cu, 595 mg; I, 9.9 mg; Fe, 7015 mg; Mn, 1600 mg; Se, 7.4 mg; Zn, 3470 mg; vitamin A, 3,000,000 IU; vitamin D3, 35,000 IU; vitamin E, 1,550 IU; vitamin K, 41 mg; Biotin, 6 mg; Folacin, 80 mg; Niacin, 950 mg; Pantothenic acid, 625 mg; Riboflavin, 175 mg; vitamin B12, 880 μg.

Table 4.2 Analyzed nutrient composition (g/kg) of diets

Nutrient	Wheat-barley-canola meal based diets		Corn-soybean meal based diets	
	High protein	Low protein	High protein	Low protein
Crude protein[1], g	195.0	160.4	226.9	190.6
Crude lipids, g	22.4	22.1	30.9	25.4
Crude ash, g	47.7	45.8	59.6	57.4
Neutral detergent fibre, g	241.2	225.7	253.8	231.0
Acid detergent fibre, g	89.5	70.7	74.5	69.9
Acid detergent lignin, g	19.1	14.0	0.0	0.0
Carbon, g	441.5	439.0	436.1	437.4
Arginine[2], g	10.5	8.0	14.5	11.9
Histidine, g	4.6	3.5	6.1	5.1
Isoleucine, g	7.3	5.4	9.7	8.1
Leucine, g	13.5	10.7	21.0	18.5
Lysine, g	8.7	8.8	11.9	10.0
Methionine, g	3.2	2.6	3.6	3.1
Phenylalanine, g	9.6	8.0	11.8	9.9
Threonine, g	6.5	6.7	8.7	7.4
Tryptophan, g	2.2	2.2	2.7	2.2
Valine, g	9.1	6.9	10.8	9.2
Non-essential amino acids, g	11.73	9.73	9.75	9.25

[1]Crude protein = N x 6.25.

[2]Amino acid analysis (Degussa, AG).

Table 4.3 Performance and nutrient retention of growing-finishing pigs fed conventional or protein-reduced diets based on wheat-barley or corn[1]

Parameter	Wheat-barley-canola		Corn-soybean meal			Effect[2], P =	
	HP	LP	HP	LP	SEM	Diet	Protein
Initial BW, kg	50.4	50.8	50.5	51.0	0.73	---	---
Final BW, kg	114.2	116.5	114.0	115.8	0.41	0.20	0.34
Gain, g/d	822.4	732.2	755.9	758.3	38.8	0.65	0.23
Feed intake, g/d	2094.2	2227.1	2075.2	2079.0	47.9	0.11	0.21
ME intake[3], MJ/d	26.2[b]	27.8[a]	28.8[a]	28.7[a]	0.6	0.01	0.23
N intake, g/d	64.5[b]	57.1[c]	75.9[a]	63.4[b]	1.5	0.001	0.001
RN[4], g/d	28.6[b]	29.6[b]	32.2[ab]	23.3[c]	1.2	0.21	0.001
RF[5], g/d	165.1	265.1	236.9	329.0	49.0	0.16	0.06
RE[6], MJ/d	10.9	14.5	13.8	16.1	1.9	0.25	0.12
Heat[7], MJ/d	15.3	13.3	15.1	12.7	0.9	0.75	0.18

[1]Values are least square means from Proc Mixed (SAS, 2005) analyses.

[2]Effects of type of diet (WBC or CS) and protein level (HP or LP) on outcome parameters.

[3]Metabolizable energy: assuming 12.5 MJ/kg for WBC and 13.8 MJ/kg for CS diets.

[4]Retained nitrogen.

[a,b,c]Different letters in a row denote differences at $P < 0.05$.

[5]Retained fat as calculated from carbon balance.

[6]Retained energy.

[7]Heat production calculated from formula Brouwer (1965).

Table 4.4 Nitrogen and carbon excretion (g/d) in feces and urine of growing-finishing pigs fed conventional or reduced-protein diets based on wheat-barley-canola or corn-soybean meal[1]

Parameter	Wheat-barley-canola meal		Corn-soybean meal		SEM	Effect, P =	
	HP	LP	HP	LP		Diet	Protein
Nitrogen							
Fecal, g/d	9.62[a]	8.67[b]	9.19[a]	8.38[b]	0.23	0.42	0.052
Urinary, g/d	26.3[a2]	18.8[b]	34.5[a]	31.7[b]	1.17	0.001	0.002
Total excretion, g/d	35.92[c]	27.47[d]	43.69[a]	40.08[b]	1.23	0.001	0.001
Carbon							
Fecal, g/d	123.6[a]	130.3[a]	97.3[a]	90.1[b]	3.5	0.001	0.98
Urinary, g/d	23.7[a]	16.9[b]	31.1[c]	28.6[c]	1.1	0.001	0.002
Total excretion, g/d	147.3[a]	147.2[a]	128.4[b]	118.7[c]	3.2	0.001	0.36

[1]Values are least square means from Proc Mixed (SAS, 2005) analyses.

[a,b,c,d]Different letters in a row denote differences at $P < 0.05$.

Table 4.5 Relationship between 4 and 24 h gas exchange measurements in growing-finishing pigs fed corn-soybean meal based diets

Parameter	4 h	24 h[1]	Relative (4 h as ratio of 24 h)
CO_2, L/d	2295[a]	2080[b]	1.11
SE	55	71	0.04
O_2, L/d	2003	1873	1.11
SE	148	233	0.09
CH_4, L/d	23.3[b]	33.6[a]	0.71
SE	3	2.4	0.09

[1]Extrapolated to 24 h measurements.

[a,b,c]Different letters in a row denote differences at $P < 0.05$.

Table 4.6 Gas exchange and greenhouse gas emission (g/d) of growing-finishing pigs fed conventional or reduced-protein diets based on wheat-barley or corn[1]

Parameter	Wheat-barley-canola meal		Corn-soybean meal		SEM	Effect[2], P =	
	HP	LP	HP	LP		Diet	Protein
CO_2, g/d[3]	1993.7[a]	1930.6[a]	1809.8[b]	1685.5[c]	53.6	0.04	0.38
CH_4, g/d[3]	25.0[a]	17.6[c]	26.0[a]	23.9[b]	1.3	0.12	0.05
CO_2-eq[4], g/d	3438.7	2948.3	3313.0	3067.7	107.7	0.98	0.09
Relative, %	100.0	85.7	100.0	92.6			

[1]Values are least square means.

[2]Effects of type of diet (WBC or CS), protein level (HP or LP).

[3]Corrected for relationship between 4 h and 24 h respiration measurements.

[4]CO_2-equivalents calculated as shown in Section 4.2.7.

[a,b,c]Different letters in a row denote differences at $P < 0.05$.

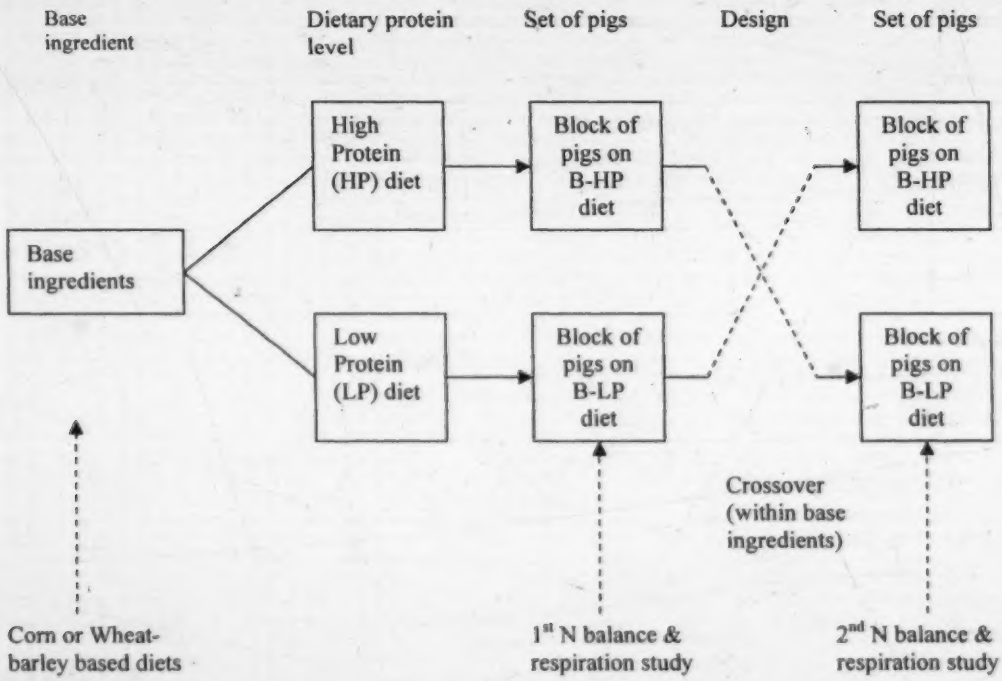
Base ingredient
Dietary protein level
Set of pigs
Design
Set of pigs
Base ingredients
High Protein (HP) diet
Low Protein (LP) diet
Block of pigs on B-HP diet
Block of pigs on B-LP diet
Block of pigs on B-HP diet
Block of pigs on B-LP diet
Crossover (within base ingredients)
Corn or Wheat-barley based diets
1st N balance & respiration study
2nd N balance & respiration study

Figure 4.1 Schematic representation of experimental design for the study, where B = base ingredient (either corn or wheat-barley).

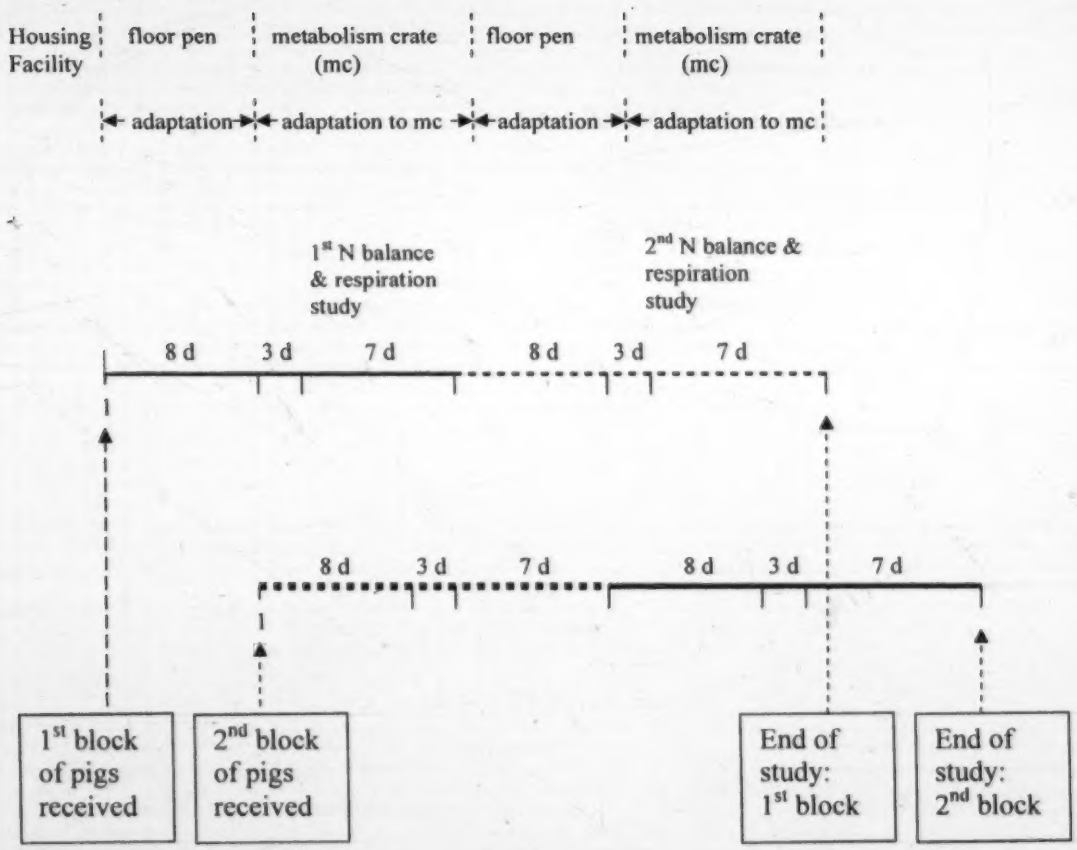

Figure 4.2 Timeline representing the housing and feeding regimen during the pre-balance, balance and respiration periods for each block of pigs.

5.0 EFFECT OF VERY LOW-PROTEIN DIETS FED TO GROWING-FINISHING PIGS ON PERFORMANCE AND GREENHOUSE GAS EMISSION

5.1 Introduction

Legislative policy and public pressure are causing pork producers to implement new technologies that reduce nitrogen, phosphorus and odor emissions, manage nutrients to prevent soil and water contamination from swine production facilities, whilst maintaining animal performance. Excess excreted nutrients and emissions from larger swine production facilities continue to draw attention as the rural-urban interface narrows and the threat of environmental impact grows. Nitrogen-containing compounds originating from intensive agriculture are contributing to a decline of forest health and species diversity in the Netherlands (van der Eerden et al., 1998) and increased nitrate concentrations in ground water in Canada (Zebarth et al., 1998).

Manipulation of swine diets to contain reduced levels of crude protein (CP) supplemented with essential synthetic amino acids (Gatel and Grosjean, 1992, Möhn and Susenbeth, 1995) has been shown to be effective at decreasing N excretion by pigs. By increasing the amount of synthetic amino acids such as lysine, methionine, threonine and tryptophan in the diet, less of protein-rich feeds such as canola and soybean meal are needed to meet the amino acid requirements of growing-finishing pigs. This reduces the amount of excess N in the diet and as a result less N is excreted. Urinary N is reduced dramatically while fecal N remains relatively constant (Hobbs et al., 1995; Kerr and Easter, 1995; Sutton et al., 1999; Whitney et al., 1998).

Many experiments have shown that the crude protein concentration of diets for growing-finishing swine can be reduced by two to three percentage units with the addition of crystalline lysine without sacrificing growth performance in corn-soybean meal diets (Hobbs et al., 1995; Kerr and Easter, 1995). Reduction of dietary protein by more than two percentage units requires addition of crystalline lysine, methionine, threonine, and tryptophan in corn-soybean meal diets (Sutton et al., 1999; Whitney et al., 1998). Reduction of dietary protein by four percentage units, with addition of lysine,

methionine, threonine, and tryptophan, resulted in a significant reduction in nitrogen excretion from growing-finishing pigs without impacting growth performance (Carter et al., 1996; Shriver et al., 2003). However, reduction of crude protein by four percentage units has also been reported to decrease daily gain and increase backfat in finishing pigs (Schoenherr, 1992; Tuitoek et al., 1997). The increase in backfat was explained by a decrease in the energy required to metabolize excess amino acids thereby increasing the net energy available for fat deposition.

Associated with dietary protein reduction are increases in carbohydrate content and decreases in non-starch polysaccharides (NSP), which originate from the protein supplements and the cereal grains. Such a change in carbohydrate content and composition may reduce CO_2 emission, and lower CH_4 and odor emission (Sutton et al., 1999; Clark et al., 2005). Carbon dioxide, on one hand, is produced *in vivo* during the oxidation of carbon-containing compounds to derive energy for metabolic processes and to create heat for maintenance of body temperature. Thus any dietary manipulation that enhances the efficiency of nutrient utilization and retention by the pig will concomitantly reduce CO_2 production and waste heat. Methane, on the other hand, is produced during hindgut fermentation of nutrients not digested and absorbed in the small intestines. Therefore, any dietary manipulation that improves small intestine digestion and absorption, limits the influx of fermentable carbohydrates (FC) or NSP into the large intestine, and thus reduces the dynamics of microbial activity in the hindgut to reduce CH_4 emission from the pigs.

Most studies involving dietary manipulation have evaluated the addition of ingredients or substances to diets to alter the microbial and/or digestive process in the gastrointestinal tract. Reduced protein diets formulated properly with supplementation of a precise balance of synthetic amino acids do not affect protein deposition of the animals (Ball and Moehn, 2003; Kerr et al., 2003; Tuitoek et al., 1997). In reduced protein, amino acid supplemented diets, the amount of amino acids in excess of the requirement of the animal (for protein deposition) is reduced, and fewer amino acids are used as energy substrate after deamination. Dietary protein reduction is achieved by partly replacing protein feeds,

such as soybean meal or canola meal, with cereal grains containing a large amount of starch (carbohydrate). Starch is more efficiently used for fat deposition than amino acids (84% vs. 52%; van Milgen et al., 2001), and starch contains 40% carbon, while amino acids contain on average 52% carbon (Schiemann et al., 1971). Therefore, reducing dietary protein content reduces carbon content.

Traditionally, nutritional research has not been concerned with GHG emissions by pigs, but was directed towards improving production efficiency. However, GHG emissions are related to nutrient efficiency in pigs. Thus, increasing the body of knowledge in the area of nutrient utilization and efficiency should provide promising directions to reducing GHG emissions.

It was hypothesized that very low protein amino acid supplemented diet (VLP) would maintain animal performance, reduce N excretion, reduce CO_2 and CH_4 emission, reduce CO_2-equivalents and lower heat production. In addition, the study was to determine nitrogen and energy balance and greenhouse gas (GHG) emission by growing-finishing pigs fed a conventional diet or a nutritionally adequate very low protein diet based on barley plus synthetic amino acids.

5.2 Materials and Methods

This experiment was approved by the Faculty Animal Policy and Welfare Committee for adherence to the Canadian Council of Animal Care (CCAC, 1993) guidelines.

5.2.1 Experimental Design

Twelve growing-finishing gilts, 55 kg initial body weight, were randomly allocated to two dietary treatments in a cross over design (Figures 5.1 and 5.2). Pig was the experimental unit, and each pig served as its own control. The pigs were adapted to diet and housing for at least 7 d. During the adaptation period, the pigs were repeatedly confined in respiration chambers to acclimatize them to the respiration boxes (234.5 cm x 106 cm x 86 cm) prior to start of respiration studies. After additional 3 d adaptation to stainless steel adjustable metabolism crates with rubber-coated expanded wire mesh

(measuring 183 cm x 61 cm x 88 cm; and 62 cm above concrete floor), 7 d nitrogen balance with quantitative collection of feces and urine was performed (Figure 5.2). Quantitative 24 h fecal collections were made as frequently as animals defecated and stored in covered plastic buckets in -20°C refrigerator until end of each daily collection period at 0600 and then frozen. At the end of a balance period the 7 d fecal collections per animal within diet and replicate were pooled, weighed and homogenized. Sub-samples (approximately 360 g) of the pooled feces were placed in aluminum pans covered by plastic bags and stored at -20°C prior to freeze-drying. After freeze drying the samples were ground with coffee grinder and stored in sealed plastic containers until chemical analyses.

Quantitative 24 h urine collections were done over 20 ml concentrated H_2SO_4 placed in special buckets with lids to prevent volatilization of urine N as NH_3 and also prevent bacterial growth, under a stainless steel collection tray covered with 0.5 mm screen preventing fecal materials from contaminating the urine collections. At end of each 24 h collection, the urine was weighed and stirred thoroughly. Then, a 5% aliquot sample (w/w) was put into brown bottles stored in a -20°C cooler till end of balance collections per animal within diet and set. At the end of entire the N balance collections the pooled aliquots of urine were weighed, thoroughly homogenized, and sub-sampled. Sub-sampled urine per animal within diet and replicate were stored at -20°C in covered plastic containers till thawed for analyses. Immediately after N balance study 4 h respiration experiment was performed using an open circuit indirect calorimetry system. The pigs were then switched to the next diet for a 7 d period of adaptation, and the N balance and respiration measurement were repeated (Figure 5.2). Animals were weighed weekly, and before and after balance periods.

5.2.2 Diet Composition

Two diets were formulated based on barley. The control, high protein (HP) diet was based on wheat, barley, canola and soybean meal, and the VLP diet containing only barley (94 % of the diet), supplemented with synthetic L-lysine, DL-methionine, L-threonine, L-tryptophan, L-isoleucine, L-valine, and L-histidine. The diets were

formulated to the same metabolizable energy content (12.55 MJ/kg), containing 17.23 % (HP) and 11.54 % (VLP) crude protein (Table 5.2). The reference diet was the gilt developer diet at the Swine Research and Technology Center (SRTC), University of Alberta (Aherne and Foxcroft, 2000). Both diets were formulated to achieve equal metabolizable energy content and equal true ileal digestible contents of lysine, methionine, threonine and tryptophan. Vitamin and mineral contents of all diets met or exceeded the recommendations of NRC (1998). The nutrient contents of the diets are shown in Tables 5.3 and 5.4.

5.2.3 Housing and Feeding

The growing-finishing pigs were received from the population of group-housed pigs at the Swine Research Unit and individually housed at the Metabolic Unit of the University of Alberta research station in two sets of six pigs. The animals were adapted to individual floor housing and diet for 7 to 10 days prior to the N balance study (Figures 5.1 and 5.2). The pigs were allowed ad libitum access to weighed quantity of feed put into self-regulating feed trough, to minimize wastage. On a daily basis, feed not eaten was collected, dried, weighed and deducted from the daily feed allowance to calculate net daily feed intake. Feed was offered twice daily, except for the respiration studies, during which half the daily allowance was offered in eight equal hourly meals to stabilize respiration rates, two hours prior to the commencement of respiration studies to facilitate feed consumption and reduce wastage in the chambers. This made it possible to monitor the activities (feeding and resting and gut motility patterns) recorded on the computer. Water was supplied ad libitum from a low pressure nipple in the low floor housing, and stainless steel adjustable metabolism crates.

5.2.4 Measurements

Body weights of the pigs were measured weekly, and before and after N balance and respiration studies on a scale (Accurate Scale Industries, Ltd., Edmonton, AB) with a digital readout (DF 2000, Massload Technologies, Saskatoon, SK).

5.2.5 Respiration Measurements

The respiration chambers (234.5 cm x 86 cm x 106 cm per chamber) consisted of commercial farrowing crates enclosed in plexiglass boxes, equipped with a feeder and low pressure nipple drinker, and a cooler to maintain temperature in the thermoneutral zone. Air was drawn through boxes via an inlet at rear and an outlet above the feed trough at rates of approximately 240 to 250 L/min. Air flow was measured after passing through a cold water condenser with commercial air meters (Canadian Meter Corp., Cambridge, Canada). A sample of air was drawn with small air pump (Gast Model 0531, Gast Mfg. Corp., Benton Harbour, MI) and delivered to O_2 (Taylor-Servomex, Crowborough, UK) and CO_2 analyzers (Beckman LB2, Beckman, Irvine, CA), and CH_4 analyzers (Qubit Systems, Kingston, ON, Canada). Air flow to the analyzers was regulated to 0.5 L/min by ball-type flow meters (Scienceware Size 2, Fisher Scientific, Mississauga, Canada). The analog output (mV) of the analyzers was converted to digital data by an analog-digital converter (Data grabber, Data Electronics, Australia) and recorded by a computer. Data acquisition was set for maximum rate (four readings per second) and the average gas concentration for each minute was recorded. For each study, the O_2 and CO_2, and CH_4 (Qubit Systems, Kingston, ON, Canada) analyzers were calibrated for zero and gain readings with either pure N_2 (zero) or calibration gas (1% CO_2, 20% O_2, and 79% N_2). Measurements of these gases at steady state and room air were recorded before and after the test period. Measurements were continuously recorded for four hours and animals were fed one quarter of their daily ration every hour. During the respiration measurements (1 h equilibration plus 4 h study) the pigs were confined in the respiration chamber. Expired air was analyzed continuously for O_2, CO_2 and CH_4 contents during the 4 h.

5.2.6 Chemical Analyses

Feed, fecal and urine samples collected were stored in a -20°C freezer prior to chemical analyses. Feed samples were taken weekly, and pooled within diet type. Pooled feed samples were analyzed for amino acids by Degussa AG, (Hanau, Germany) using ion exchange chromatography (Llames and Fontaine, 1994). Tryptophan was analyzed by fluorescence detection HPLC after alkaline hydrolysis with barium hydroxide

octahydrate at 110°C for 20 h (Fontaine et al., 1998). Gross energy of feed and fecal samples was determined using an adiabatic bomb calorimeter (Leco AC-300, LECO Corp., St. Joseph, MI). Urine N content was determined according to macro-Kjeldahl (AOAC, 1998). Proximate analyses of feed, and fecal samples involving dry matter, crude protein (macro-Kjeldahl, AOAC, 1998), ash, ether extract (Goldfisch extraction apparatus, Labconco Corp., Kansas City, MO), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Ankom Fiber Technique, Goering and van Soest, 1970) were determined on feed and fecal samples. Total carbon content of feed and freeze-dried fecal samples ground in a MM2 Retsch/Birkmann mixer mill (Brinkmann Instruments Inc., Westbury, NY), were analyzed using a NA1500 Carlo-Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ). Prior to proximate analyses, feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a 1.0 mm screen

5.2.7 Calculations

During the nitrogen balance period, whole tract digestibility of nitrogen, phosphorus, calcium, ether extracts, NDF, ADF and gross energy were determined. Lipid and energy retention were calculated according to C-N balance technique, assuming 23.8 kJ/g deposited protein, and 39.6 kJ/g deposited lipid (Noblet et al., 1987; Moehn and Susenbeth, 1995). The amount of digested nutrients and energy, and nitrogen and energy excretion with urine, was measured. Digested protein (DP) and fat (DF) and carbohydrate (DCHO) expressed in energy units were calculated from the 7d balance experiments as:

DP, kJ = diN x 6.25 x 23.86, kJ/g, where diN (g) = digested nitrogen

DF, kJ = diF, g x 39.76, kJ/g, where diF (g) = digested fat

DCHO, kJ = DE, kJ – DP, kJ – DF, kJ, where DE = digestible energy.

Maintenance net energy (NEm) was taken as the intercept of a regression of heat production on feed intake (per $kg^{0.75}$ body weight, Noblet et al., 1987), at zero growth. Dietary net energy (NE) was calculated as retained energy (RE) plus maintenance net energy (NEm).

After determination of NDF, ADF and ADL values for feed and fecal samples, using the modified method of Goering and van Soest (1970) hemicellulose was calculated as difference between NDF and ADF. Cellulose content of feed and feces was calculated as difference between ADF and ADL.

The set of factors and constants from (Brouwer, 1965) was used in all calculations. Heat production (HE) based on the measurements of gas exchange and 7-d determination of nitrogen excreted in urine (U_N) was calculated as:

$HE\ (kJ) = 16.18 * V_{O2} + 5.02 * V_{CO2} - 5.99 * U_N - 2.17 * V_{CH4}$ (Brouwer, 1965).

Retained (or mobilized) energy in protein (RP) was calculated from the nitrogen balances and gas exchange measurements as:

$RP, kJ = (diN - U_N) \times 6.25 \times 23.86, kJ/g$

Retained (or mobilized energy in fat (RF) was calculated either as:

RF, kJ = ME, kJ – HE, kJ – RP, kJ, where ME = metabolizable energy (kJ); HE = heat production (kJ); RP = retained energy in protein (kJ), assuming no retention of carbohydrate.

The RP and RF were also calculated from carbon (C) balances as:

C in CO_2 emitted, g = CO_2 production, litres x 0.536, g/L

C in RP, g = RP, g x 0.52, where 0.52 is average C content in protein

C balance, g = C intake, g – (C in feces, g + C in urine, g + C in CO_2, g)

C in RP, g = C balance, g – C in RF, g

RF, kJ = C in RF, g/0.767 x 39.76, kJ/g, where 0.767 is average C content in fat.

Carbon (C) retention was calculated as:

$C_{intake} - C_{in\ feces} - C_{in\ urine} - C_{in\ CO2} - C_{in\ CH4}$.

The carbohydrate content of the animals was assumed to remain constant during the respiration period. The C content taken as: 12 g/mol for CO_2 and CH_4, and as 90% of the N content of urine (Schiemann et al., 1971). Lipid retention calculated as:

$C_{retention}$ minus $C_{retained}$ in protein (52%) divided by the C content in body fat (76.7%), as reported by Schiemann et al. (1971).

Metabolizable energy (ME) intake was calculated as:

ME, kJ = DE – CH_4E – UE, where DE = digestible energy (kJ/g); CH_4E = energy in CH_4 (39.56 kJ/L; Schiemann et al., 1971); UE = energy in urine (UE, kJ/g = 0.333 * % C + 0.093 * % N, Hoffmann and Klein, 1980). RE taken as energy retained as protein and fat (22.6 kJ/g and 39.6 kJ/g respectively; Böhme and Gädecken, 1980).

Respiratory quotient (RQ), based on the measurements of gas exchange and 7-d determination of nitrogen excreted in urine (U_N) was calculated as:

$RQ = (V_{CO2} - [U_N \times 6.25 \times 0.774])/(V_{O2} - [U_N \times 6.25 \times 0.957])$, where V_{CO2} = volume of CO_2 emission (litres); V_{O2} = volume of O_2 consumption (litres); U_N = urinary nitrogen (g). In its simplistic form the equation for RQ is expressed as: $RQ = V_{CO2}/ V_{O2}$. The RQs were calculated to determine the types of substrate utilized by the growing-finishing pigs during the study period.

CO_2-equivalents, from the growing-finishing pigs, were calculated using factors of the global warming potential (GWP): 1, 21 and 310 for CO_2, CH_4 and N_2O respectively, on a molar basis (Grubb et al., 1999; IPCC, 1997). The equations were as follows:

CO_2-eq (CO_2) = CO_2, d, where CO_2, d = CO_2, g x 1440/1.114.

CO_2-eq (CH_4) = 21 x 44/16 x CH_4, d, where CH_4, d = CH_4, g x 1440/0.627.

CO_2-eq from pigs (CH_4+ CO_2) = 21 x 44/16 x CH_4, d + CO_2, d.

The conversion of nutrients in manure to greenhouse gases is not well established, therefore, the range in CO_2-equivalents from manure were calculated using estimated conversion rates of 5 or 30% of the N in manure to N_2O (Béline et al., 1999), and 3 or 20% of C in manure to CH_4 (Martinez et al., 1999). The contents of N and C in manure were taken as the excreted N and C by the pigs. Total excretion values (TN and TC respectively) per pig were calculated as the sum of fecal and urinary N and C excretion. Lipid and energy retention were calculated using the C-N balance technique.

5.2.8 Statistical Analyses

The effects of type of diet and protein level were estimated using the Proc mixed model procedures (SAS, 2005). The interaction between type of diet and protein level was tested for all outcome parameters and retained in the model if $P < 0.10$. Least squares means (LSM) were calculated and the probability of differences between treatments determined with the 'pdiff' option, which performs a pair-wise comparison based on a two-tailed t-test. Significance was set as $P < 0.05$, and all P-values between 0.05 and 0.1 were regarded as trends.

5.3 Results

The chemical composition of the diets was in agreement with the objectives of the experiment and met the dietary protein reduction of 6 percentage units (Tables 5.3 and 5.4). In general, analyzed values were close to formulated values. Lysine and other AA concentrations were very close to targeted values. Cell wall fractions (i.e. NDF and ADF) were lower for the very-reduced protein, AA supplemented diet. This indicates changes in fermentable carbohydrate composition associated with the reduction of dietary protein concentration. Protein and energy metabolism, based on 24 h gas exchange, was measured in 4 balance experiments with 24 gilts in the live weight range 50-110 kg. The pigs were measured alternately on HP and VLP diets, at ad libitum (Figures 5.1 and 5.2).

5.3.1 Animal Performance

Data given in Table 5.5 indicate that, during the 7-d collection period final body weight, body weight gain, average daily feed intake, feed efficiency and intake of metabolizable energy were not affected ($P > 0.1$) by protein level in both diets. The differences in nitrogen retention, energy retained in protein, efficiency of utilization of energy for tissue accretion and metabolizable energy for maintenance (MEm) from the HP versus VLP were concomitantly not significant ($P > 0.1$). The difference in MEm due to the diet (HP vs. VLP) was no significant. Thus retained fat and energy retention in fat were higher ($P = 0.08$) for VLP than HP (Table 5.5).

5.3.2 Oxygen Consumption, and CO_2 and CH_4 Emission

Oxygen intake was lower ($P = 0.04$) due to protein effect (1557 g/d, VLP vs 1823 g/d, HP). The relative reduction was 15%. Emissions of CO_2 and CH_4 were reduced ($P = 0.07$; Table 5.6) due to efficient utilization of carbon skeletons in carbohydrates than in protein. These reductions support the theory that formulation of diets close to the ideal protein by reducing dietary protein while adding amino acids as the method of choice to reduce N excretion. Associated with such protein reduction: (1) Lowering protein concentration increases carbohydrate (CHO) content in the LP diet; (2) The expected change in CHO content and composition may reduce CO_2 emission and (or) affect CH_4 emission. The reductions also support our hypothesis that improving nutrient utilization will reduce emissions (or excretions) from pigs. The corresponding respiratory quotients (1.25, VLP vs 1.14, HP) fall within the range of literature values.

5.3.3 Energy metabolism

Metabolizable energy for maintenance (12.34 MJ, VLP vs 12.22 MJ, HP SEM 0.44) was not affected ($P > 0.1$) by dietary protein reduction (Table 5.6). Net energy, (calculated by as MEm + RPE + RFE divided by daily feed intake) was increased ($P = 0.02$). The effect of VLP on net energy (15.99 MJ, VLP vs 10.53 MJ, HP SEM 1.94) was a trend ($P = 0.06$). Heat production was calculated based on C-N method, RQ method and from Brouwer formula (a combination of C-N and RQ methods). Heat production estimates as HE (CN) and HE (Brouwer) were significantly lower ($P < 0.05$) for VLP fed pigs. Heat production (MJ/kg) was not affected ($P > 0.1$), but a similar pattern to HE (kJ/min), by protein reduction. The reduction in heat increment approached significance ($P = 0.06$; Table 5.6).

5.3.4 Nutrient Intake and Excretion (N and C)

Results of the N balance indicate a clear reduction in N intake and total N excretion ($P = 0.0001$; Table 5.7) due to protein effects. The relative reductions were 34 and 48%, respectively. These reductions in N intake and excretion did not affect gain achieved with the very-reduced protein diet, although there appeared to be a fair balance of the amino acid ratios in relation lysine for maintenance and protein accretion (NRC, 1998).

Carbon intake and excretion were not affected ($P > 0.1$) by reduction in protein concentration (Table 5.7). Carbon retention showed an increased trend ($P = 0.06$) due to the protein effect in agreement with the pattern for energy retained in fat and fat retention.

5.3.5 Gas Exchange and GHG Emission

The effect of very-reduced protein, AA-supplemented diets during the respiration experiments indicate that oxygen consumption (0.85 L, VLP vs. 1.04 L, HP; SEM 0.04) was lower (18.3%) for pigs fed the VLP diet ($P = 0.02$). Carbon dioxide emission (0.81 L, VLP vs. 0.86 L, HP; SEM 0.02) was reduced (5.8%) by VLP ($P = 0.02$; Table 5.6). The respiration quotient (0.95, VLP vs. 0.83, HP) was higher (14.5%). Methane emission (0.011 L, VLP vs. 0.014 L, HP; SEM 0.001) was reduced (21.4%) by pigs fed VLP diet ($P = 0.07$). Consequently, greenhouse gas emission by pigs (CO_2-equivalents) was reduced by 10.5% (2.98 kg, VLP vs. 3.33 kg, HP; SEM 0.23) (Table 5-8). Heat production (18.4 kJ, VLP vs. 21.2 kJ, HP; SEM 0.9), calculated by gas exchange, was lower (13.2%) for pigs fed VLP diet ($P = 0.04$; Table 5-6).

5.4 Discussion

The objectives of this study were to determine nitrogen and energy balance, and greenhouse gas emission by growing-finishing pigs fed HP or a nutritionally adequate, VLP diet based on barley supplemented with eight synthetic amino acids. It was hypothesized that very-low protein, amino acid supplemented diet would maintain animal performance, reduce N excretion, reduce CO_2 and CH_4 emission, reduce CO_2-equivalent and lower heat production. The dietary CP contents were reduced by 6 percentage units (with supplementation of L-lysine HCl, DL-methionine, L-threonine, L-tryptophan, L-valine, L-histidine, L-isoleucine, and L-leucine) in the VLP diet. The available phosphorus, which averaged averaged 3.2 g/kg in both diets, was also optimal.

Reducing CP content of the diet with AA supplementation reduces excess total N intake and, thus, has been reported to dramatically decrease N excretion. A 2-percentage unit

decrease in dietary CP with lysine supplementation has been reported to decrease N excretion by 17 to 22% (Cromwell and Coffey, 1993). A more profound decrease in N excretion is observed when CP content of the diet is reduced by 3 to 4 percentage units with appropriate AA supplementation (Sutton et al. (1996, 1999; Shriver et al., 2003). Carter et al. (1996) reported that N excretion could be reduced in growing and finishing pigs by approximately 35% by reducing CP concentration with supplementation of lysine, methionine, threonine, and tryptophan. Our finding on reduction in N excretion (48%, relative) was greater than that of Sutton et al. (1996; 1999) and Shriver et al. (2003) who reported a 28% and 27.3% reduction, respectively, in N excretion from pigs fed low-protein diets.

Reducing dietary protein maintained performance and reduced nutrient excretion. The performance of the growing-finishing pigs given nitrogen-reduced diets and balanced with adequate amounts of industrial amino acids is in agreement with published results (Spiekers et al., 1991; Valaja and Alavinhkola, 1993; Kay and Lee, 1996). Further, as was examined with these feeding regimen, nitrogen excretion per unit weight gain could be decreased by over 35% without negatively impacting performance (Table 5.7). These results are in accordance with findings described by in the literature (Kirchgessner and Roth, 1993; Bourdon et al., 1995; Bridges et al., 1995; Kay and Lee, 1996; Nonn et al., 1997; Canh et al., 1998). On the contrary, supplementing similar synthetic amino acids Kendall et al. (2000), Schoenherr (1992), and Tuitoek et al. (1997a, b) observed reduced performance of finisher pigs. In other studies reported in the literature, protein reduction was achieved by replacing protein-bound ingredients like soybean meal with appropriate amounts of limiting amino acids such as lysine, methionine, threonine or tryptophan, but observed limited performance (Gatel and Grosjean, 1992; Lee et al., 1993; Lenis and Jongbloed, 1999; Roth and Kirchgessner, 1993).

In agreement with results of Dourmad et al. (1993), N excretion in growing-finishing pigs can be reduced by about 10% for each percentage-point CP reduction in the feed. In agreement with studies of Le Bellego et al. (2001), Dourmad et al. (1993), Tuitoek et al. (1997a) and Canh et al. (1998), our results demonstrate that reduction of CP level, while

maintaining essential AA in the feed, does not affect the N retention by the animals. Similarly, the reduction of retained nitrogen and (or) energy retention for low-CP diets previously demonstrated by Quiniou et al. (1995), can be attributed to the balance technique. Indeed, this technique underestimates N losses and consequently overestimates N retention, and the overestimation becomes more important as CP level in the diet or N losses are elevated. This observation also emphasizes the inadequacy of the balance technique for estimating N retention at variable CP intakes. Such explanation is supported by growth performance of pigs in this study. Thus, when VLP diets are balanced in terms of essential AA and adequate for ratios between energy and protein supply, it is feasible to reduce CP levels by 6% without affecting N retention, lean tissue growth or BW gain.

The fat retention and energy retained in fat tended to be greater ($P = 0.08$) for the VLP than the HP diets. This means that N retention was limited by intake of an amino acid from the VLP diets. Thus energy was retained as fat rather than used to synthesize more protein. Our results are agreement with the findings of Quiniou et al. (1995), in a study comparing two measurement methods to evaluate effect dietary CP level on protein and energy balance. In their study low-protein fed pigs retained more energy as fat, and less energy as protein than HP pigs.

Reduced protein diets have been associated with a reduction in energy losses (Fuller et al., 1987; Noblet et al., 1987; Roth et al., 1999). Therefore, reducing CP increases the energy available for tissue deposition. Our results showed reduced heat production by pigs fed VLP compared to HP diet, irrespective of method used for the calculation. The reductions in heat production (kJ/min), based on the C-N and Brouwer formula were significant ($P < 0.05$). The reduced heat production consequently resulted in lowered heat increment associated with dietary protein reduction. Energy metabolism and substrate oxidation measured by means of indirect calorimetry and nutrient balance.

The RQ value of 1.25 (VLP) and 1.14 (HP) indicate that during the feeding period, dietary carbohydrates were the main energy source, sufficient to cover energy

requirement without oxidation of fat (Chwalibog and Thorbek, 2000; Chwalibog et al., 2004). Such RQ values, averaging 1.2, compare favourably with values obtained during 24 h respiration studies (Chwalibog et al., 2004). Under normal feeding conditions, the main source of energy for growing-finishing pigs is dietary carbohydrates followed by protein, and provided there is enough energy from carbohydrate and protein to sustain requirements for maintenance and growth, dietary fat is not oxidized but retained in the body (Chwalibog and Thorbek, 2000). Our results are in agreement with this hypothesis. However, during starvation mobilized body fat becomes the main source of energy followed by body protein oxidation. This situation is changed during re-feeding as macronutrients are again available from the diet (Chwalibog et al., 2004). Consequently, the metabolism will change from utilizing body fat and protein reserves to using dietary nutrients. The switch between endogenous and exogenous substrates is not an immediate process and a certain time is necessary to re-establish metabolism from catabolic to anabolic conditions (Chwalibog and Thorbek, 2000; Chwalibog et al., 2004). This assumes that the climate in the respiration chambers was kept constant at a temperature of 22°C and a relative humidity of 60%.

Also, in our calculations we do not describe individual pathways of nutrient metabolism, but the general relations between substrates and product. The calculations were carried out in accordance with the method described in Section 5.2, using constants and factors generally accepted for studies in energy metabolism in animals (Brouwer, 1965). Our results are based on measurement of oxygen consumption and CO_2 emission by indirect calorimetry and urinary nitrogen excretion.

Calculations of energy expenditure and substrate oxidation are sensitive to the accuracy of measurements of gas exchange (Jebb et al., 1996) and can be biased by the potential pitfalls in the interpretation of data from gas exchange measurements (Elia and Livesey, 1988; Ferrannini, 1988; Chwalibog and Thorbek, 2000). However, the accuracy of the gas exchange measurements was high in our experiment with an overall error of less than ±1.5%. In addition, errors in collection of urine and unaccounted for evaporation of

ammonia may cause an underestimation of the urinary nitrogen and consequently heat production may be underestimated.

Contrary to calculations of other researchers who have done similar work where retentions are calculated simply as intake minus excretion (for the specific nutrient in feed and feces and (or urine), our calculation for C excretion accounted for C in feed, feces, urine, CO_2 and CH_4 quantitatively. Over all, reducing the dietary protein reduced N excretion, CO_2 and CH_4 emission (g/d) by pigs. The CO_2-equivalents arising from the pigs (animals) themselves – from CO_2 and CH_4 production – was significantly lower with very-reduced protein diet. The reduction in CO_2 and CH_4 emission (g/d) is in agreement with the calculated and analyzed NDF and ADF (i.e., fermentable carbohydrate or fibre) contents of diets in relation to the reduced N and change in diet carbohydrate content and composition, in accordance with the hypothesis and objectives (i.e. reduced CO_2, CH_4, CO_2-equivalent and heat production). The results indicate enhanced nutrient utilization.

Further GHG emission will depend on the conversion of the carbon and N excreted into manure into CO_2, CH_4, and N_2O. Our results show that reducing dietary protein intake reduces the N and C excretion by pig and has the potential to reduce GHG emissions by pigs by 10%. To realize the full potential, the conversion of nutrients in manure into GHG must be investigated further, along with means to influence this conversion.

Energy metabolism and substrate oxidation were measured by means of indirect calorimetry and nutrient balance (Chwalibog et al., 2004). The RQ values (volume/volume) (0.95, VLP vs 0.83, HP) were higher than for fat oxidation. The values, however, indicate that during the feeding period, dietary carbohydrates were the main energy source, sufficient to cover energy requirement without oxidation of fat (Table 5.8). Such RQ values compare favorably with values obtained during 24 h respiration studies (Chwalibog et al., 2004). Under normal feeding conditions, the main source of energy for growing-finishing pigs is dietary carbohydrates followed by protein, and provided there is enough energy from carbohydrate and protein to sustain requirements for maintenance and growth dietary fat is not oxidized but retained in the body

(Chwalibog and Thorbek, 2000). However, during starvation mobilized body fat becomes the main source of energy followed by body protein oxidation. This situation is changed during re-feeding as macronutrients are again available from the diet. Consequently, the metabolism will change from utilizing body fat and protein reserves to using dietary nutrients. The switch between endogenous and exogenous substrates is not an immediate process and a certain time is necessary to re-establish metabolism from catabolic to anabolic conditions. This assumes that the climate in the respiration chambers was kept constant at a temperature of 22°C and a relative humidity of 60%. It is also assumed that our calculations do not describe individual pathways of nutrient metabolism but the general relations between substrates and product. The calculations were carried out in accordance with the method described in Section 5.2, using constants and factors generally accepted for studies in energy metabolism in animals (Brouwer, 1965). Our results are based on measurement of oxygen consumption and CO_2 emission by indirect calorimetry and urinary nitrogen excretion. Calculations of energy expenditure and substrate oxidation are sensitive to the accuracy of measurements of gas exchange (Jebb et al., 1996) and can be biased by the potential pitfalls in the interpretation of data from gas exchange measurements (Elia and Livesey, 1988; Ferrannini, 1988; Chwalibog and Thorbek, 2000). However, the accuracy of the gas exchange measurements was high in our experiment with an overall error of less than ±1.5%. In addition, errors in collection of urine and unaccounted for evaporation of ammonia may cause an underestimation of the urinary nitrogen and consequently heat production may be underestimated.

In recent papers the theoretical basis of indirect calorimetry in research with humans in health and disease has been discussed (Elia and Livesey, 1988; Ferrannini, 1988; Livesey and Elia, 1988). By indirect calorimetry the heat production (HE) can be calculated either by the respiratory quotient (RQ) method (HE_{RQ}) or by the C-N balance method (HE_{CN}). Both methods are reliable and can be carried out with high accuracy, as discussed by Christensen et al. (1988). The RQ method is based on measurements of O_2 consumption, CO_2 and CH_4 emission and the amount of N excreted in the urine. The HE from oxidation of non-protein materials (glucose and triacylglycerol) is based on their respective RQ values (V_{CO2}/V_{O2}) and the heat released during complete oxidation. The validity of

indirect calorimetry when RQ is above 1.0, associated with lipogenesis, has been demonstrated by Elia and Livesey (1988). In humans RQ values above 1.0 have been obtained during infusion of glucose (Askanazi et al., 1980) and high-carbohydrate meals (Acheson et al., 1984). In animals RQ values above 1.0 were measured in dogs by over-feeding with glucose over a 4 h period (Lusk, 1928, cited in Jakobsen and Thorbek, 1993) and in geese by overfeeding with carbohydrates (Benedict and Lee, 1937, cited in Jakobsen and Thorbek, 1993). In growing-fattening pigs on high feed intake RQ values, measured in 24 h periods, were constantly above 1.0 (Thorbek et al., 1984; Christensen, 1985). HE_{CN} is calculated as the difference between metabolizable energy (ME) and energy retained in fat + protein (RE), measured by C-N balances. Note, our figures are slightly higher because unlike the previous studies we accounted for C excreted into CH_4 too (see calculations section)]. In measuring the C-N balances the amount of total fat retention can be calculated directly (Christensen et al., 1988), while fat retention by the RQ method is calculated indirectly as the difference between retained energy (determined as $RE = ME - HE_{RQ}$) and the energy retained in protein. Jakobsen and Thorbek (1993) observed that RQ was linearly related to the amount of fat retention measured by the C-N balances.

The calculations of energy metabolism were carried out with constants and factors generally accepted in energy metabolism studies (Brouwer, 1965). The logic of the calculations is based on the overall model of nutrient metabolism, which does not describe intermediary pathways but the general relations between substrates and products. The utilization of nutrients for catabolic and anabolic processes was evaluated in terms of energy transfer. Another assumption that causes differences in the literature values is that urinary energy from protein metabolism is on average 24.9 kJ/g U_N (Nehring et al., 1965) for pigs, but these values can vary depending on the composition of protein metabolites in urine. Furthermore, short-term changes of glycogen content in the body were not considered, because the results are based on long-term measurements (24 h) from which it was expected that glucogenesis and glycolysis were in balance.

The amounts of oxygen consumed to oxidize 1 g of starch, 1 g of fat or 1 g of protein in the body are 1.184 g, 2.875 g and 1.366 g, respectively (Brouwer, 1965). Hence oxygen consumption is predicted as:

$M_{O2} = 1.184\ (dcf + nfe) \times I_f + 2.875\ (dcl \times I_f - G_l) + 1.366\ (dcp \times I_f - Gp)$, where M_{O2}: oxygen consumption (kg/d), I_f: feed intake (kg/d), dcf: digestible crude fibre (kg/kg), dcp: digestible crude protein (kg/kg), G_l: fat retention (kg/d) and Gp: protein retention (kg/d). The amount of CO_2 emitted from the oxidation of 1 g starch, 1 g fat or 1 g protein in the body, are, respectively, 1.629 g, 2.81 g and 1.52 g (Brouwer, 1965). Therefore, the CO_2 emission can be expressed as:

$M_{CO2} = 1.629\ (dcf + nfe) \times I_f + 2.81\ (dcl \times I_f - G_l) + 1.52\ (dcp \times I_f - Gp)$, where M_{CO2}: carbon dioxide production (kg/d). The heat production of livestock according to Brouwer (1965) is:

$HE = 16.18 * V_{O2} + 5.02 * V_{CO2} - 2.17 * V_{CH4} - 5.99 * U_N$, where HE: heat production (MJ), V_{O2}: oxygen consumption (m^3), V_{CO2}: carbon dioxide emission (m^3), V_{CH4}: methane emission (m^3) and U_N: nitrogen excretion with urine (kg) and the respiration quotient (volume of CO_2 emitted divided by volume of O_2 consumed) is: $RQ = V_{CO2} / V_{O2}$ or $V_{O2} = V_{CO2}/RQ$. Rearranging the RQ equation, ignoring the terms of methane and nitrogen excretion with the urine (Brouwer, 1958) gives: $HE = (16.18\ V_{CO2}/RQ) + 5.02\ V_{CO2}$ or $HE = [(16.18/RQ) + 5.02] \times V_{CO2}$.

The literature contains few references related to the respiratory quotient in pigs. Theoretically, RQ ranges from 0.71 to 1.3 (Brouwer, 1957) depending on metabolic rate, feed intake and individual status of the animals. If livestock are fed poorly, the RQ will be low; it increases with feed intake. The oxygen consumption and CO_2 emission of livestock are generally measured in respiration chambers, as described by Verstegen et al. (1987). Most publications do not present any RQ, but give heat production calculated from respiration experiments. The RQ can be seen as a reflection of the kind of substrate of the feed that is being oxidized. RQ values varying from 0.8 to 1.2 are found in cattle. Sechen et al. (1989) found an RQ of 1.01 for lactating dairy cows. Schrama et al. (1993) found RQ values of 0.79 to 0.81 for poorly fed young calves.

Feddes and DeShazer (1988) referred to an RQ of 1.1. For growing-fattening pigs the RQ ranged from 0.96 to 1.05 at a low feed level, but from 1.03 to 1.16 at a high feed level (Thorbek et al., 1984). Feddes and DeShazer (1988) reported 1.1 for pregnant sows at high metabolic rate. Suggested respiratory quotients for different classes of livestock and different physiological status (van Ouwerkerk et al., 1994) are shown in Table 5.9.

5.5 Conclusion

Overall, the results of this experiment indicate that diets formulated exclusively on barley supplemented with appropriate amino acids can be fed to growing-finishing pigs. Feeding VLP diet maintained animal performance, reduced N excretion, reduced heat production (indicator of improved energy utilization). Also, such dietary manipulation can reduce GHG production by pigs.

The core methodology is based on a combination of nitrogen and energy balances with indirect calorimetry. This makes it possible to estimate protein, fat and energy retention and mobilization in the intact whole body (*in vivo*), total heat production and net substrate oxidation, as well as to calculate energy transfer between protein, carbohydrate and fat at the whole body level.

By combining data from gas exchange measurements with nitrogen and energy balances it is possible to evaluate the contribution of nutrients to the oxidative processes and energy transfer between substrate pools. Consequently, data from numerous balance and respiration experiments can be incorporated to design a nutritional model and provide quantitative description of nutrient metabolism under a variety of nutritional, genetic and environmental conditions.

5.6 Implication

Reducing crude protein concentration of the diet of growing-finishing pigs with amino acid supplementation can markedly reduce nitrogen excretion. Swine producers looking for alternatives to reduce the amount of nitrogen excreted from swine should consider the use of very-reduced protein, amino acid supplemented diets.

The public concern related to the potential for water and air pollution from swine facilities demands that alternative management practices be developed. Dietary manipulation offers a potential method to reduce nutrient excretion by swine. Results from our experiments suggest that lowering crude protein by 6 percentage units with addition of amino acids can reduce total nitrogen excretion. There is the possibility that pork producers could sell their reductions in GHG emissions as GHG credits to organizations that are not able to reduce their own emissions adequately. Carbon credits are valued at $15 – 60 per tonne (Ball and Moehn, 2003). A quantitative model describing the concomitant relationships between nutrient oxidation, protein retention and liponeogenesis from carbohydrates for pigs and poultry (and other animal species) could be evolved. Data from the gas exchange and respiration quotients could be used in modeling and evaluating ventilation rates in livestock buildings.

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Table 5.1 Effect of replacing one gram protein by one gram starch[1]

Carbon involved	Protein	Starch
C intake, g	0.52	0.40
C into fat[2], g	0.27	0.34
C into CO_2, g	0.25	0.06
CO_2 production[3], g	0.92	0.22
Difference, g	0.70	

[1]Calculations based on literature (Schiemann et al., 1971, van Milgen et al., 2001).

[2]Quantitative use of carbon for fat synthesis

[3]g C x 12/44; 1 mol CO_2 (44 g) contains 12 g C.

Table 5.2 Ingredient composition (kg/tonne) of control (HP) and very-reduced protein, amino acid supplemented (VLP) experimental diets[1]

Ingredient	HP	Very low protein
Barley	500.00	941.85
Wheat	290.00	0
Canola meal	75.00	0
Soybean meal	100.00	0
Canola oil	10.00	26.50
L-Histidine	0	0.05
L-Isoleucine	0	0.41
L-leucine	0	0.24
L-Lysine HCl	0	4.05
DL-Methionine	0	0.19
L-Threonine	0	1.37
L-Tryptophan	0	0.10
L-Valine	0	0.24
Premix (Breeder #4)[2]	25.00	25.00

[1]Diets formulated on as fed basis and met requirements (NRC, 1998).

[2]Provided per kilogram of premix: Ca, 21.5%; available P, 8.4%; Na, 4.8%; Mg, 1%; Cu, 595 mg; I, 9.9 mg; Fe, 7,015 mg; Mn, 1,600 mg; Se, 7.4 mg; Zn, 3,470 mg; vitamin A, 3,000,000 IU; vitamin D3, 35,000 IU; vitamin E, 1,550 IU; vitamin K, 41 mg; Biotin, 6 mg; Folacin, 80 mg; Niacin, 950 mg; Pantothenic acid, 625 mg; Riboflavin, 175 mg; vitamin B12, 880 μg.

Table 5.3 Calculated nutrient composition (g/kg) of experimental diets

Nutrient, g/kg	High protein	Very low protein
Crude protein	172.30	115.4
Histidine	3.64	2.07
Isoleucine	5.74	3.57
Leucine	10.82	6.56
Lysine	6.14	6.14
Methionine	2.52	1.73
Threonine	5.05	4.26
Tryptophan	1.82	1.15
Valine	12.7	6.0
Phosphorus (avail.)	3.30	3.09
Neutral detergent fiber	169.5	158.4
Acid detergent fiber	64.9	58.4
ME, MJ/kg	12.55	12.55

Table 5.4 Analyzed nutrient composition (g/kg) of experimental diets

Nutrient, g/kg	High protein	Very low protein
Dry matter	899.5	894.0
Crude protein[1]	181.6	120.4
Ether extract	22.9	42.4
Crude ash	44.8	42.9
Neutral detergent fiber	175.6	164.5
Acid detergent fiber	70.3	69.8
Acid detergent lignin	na[3]	na
Carbon	427.8	440.5
Essential amino acids[2]		
Arginine	11.2	5.9
Histidine	4.3	2.6
Isoleucine	6.9	4.6
Leucine	12.7	8.5
Lysine	8.0	7.6
Methionine	3.1	2.3
Phenylalanine	8.8	6.3
Threonine	6.6	5.4
Tryptophan	na[3]	na
Valine	12.7	6.0
Non-essential amino acids	na	na

[1]N x 6.25, determined with macro-Kjeldahl (AOAC, 1998).

[2]Amino acid analysis by Degussa, AG.

[3]Not available

Table 5.5 Effect of very-reduced protein amino acid supplemented diet on performance of growing-finishing pigs fed ad libitum[1]

Item	High protein	Very low protein	SEM	Significance[2], *P* =
No. of animals	12	12		
Average initial BW, kg	56.37	55.15	7	---
Average final BW, kg	98.93	102.74	18	NS
Feed intake, g/d	2278	2287	79	NS
Gain, g/d	828	819	24	NS
Gain : Feed	0.36	0.37	0.01	NS
ME intake, MJ/d	28.38	28.90	1.43	NS
RN[3], g/d	21.7	20.9	1.5	NS
RF[4], g/d	250.40	379.22	49.8	0.08
RPE[5], MJ/d	3.23	3.12	0.23	NS
RFE[6], MJ/d	9.02	13.65	1.79	0.08
kpf[7]	0.80	1.49	0.33	NS
MEm[8]	12.22	12.34	0.44	NS

[1]Values are least square means.

[2]Effects of type of protein level (HP or VLP) on outcome parameters.

[3]Retained nitrogen.

[4]Retained fat.

[5]Retained energy in protein.

[6]Retained energy in fat.

[7]Efficiency of tissue deposition = (RPE + RFE)/($Heat_{CN}$ – MEm).

[8]Metabolizable energy for maintenance = 0.458 x $BW^{0.75}$.

Table 5.6 Effect of very-reduced protein amino acid supplemented diet on energy, gas exchange and heat production of growing-finishing pigs fed ad libitum[1]

Item	High protein	Very low protein	SEM	Significance[2], P =
No. of animals	12	12		
MEm[3]	12.22	12.34	0.44	NS
NE[4]	10.72[a]	12.13[b]	0.48	0.02
NEbr[5]	10.53	15.99	1.94	0.06
O_2 consumption, g/d	1822.73[a]	1556.50[b]	83.75	0.04
CO_2 emission, g/d	2081.77	1942.98	79.27	0.07
CH_4 emission, g/d	21.77	17.60	3.25	0.07
RQ[6]	1.14	1.25	---	---
Heat (CN)[7], kJ/min	16.02[a]	13.18[b]	0.97	0.02
Heat (RQ)[8], kJ/min	0.0167	0.0145	0.0008	0.07
Heatbr[9], kJ/min	21.20[a]	18.43[b]	0.77	0.04
Heat, MJ/kg	30.07	27.06	1.42	NS
Heat increment	17.85	14.27	1.30	0.06

[1]Values are least square means.

[2]Effects of type of protein level (HP or VLP) on outcome parameters.

[3]Metabolizable energy for maintenance = 0.458 x $BW^{0.75}$.

[4]Net energy = (MEm + RPE + RFE)/(0.001 x feed intake).

[5]Net energy calculated based on formula (Brouwer, 1965) = ME intake – Heat – MEm.

[6]Respiration quotient = Volume of CO_2 emission/ Volume of O_2 consumption.

[7]Heat production based C-N method

[8]Heat production based on RQ

[9]Heat production calculated from Brouwer (1965).

[a,b]Different letters in a row denote differences at $P < 0.05$.

Table 5.7 Effect of very-reduced protein amino acid supplemented diet (g/d) on intake and utilization of N and C in growing-finishing pigs fed ad libitum[1]

Item	High protein	Very low protein	SEM	Significance[2], P =
No. of animals	12	12		
Nitrogen				
N intake	65.72[a5]	44.38[b]	2.6	0.0001
Relative[3]	100	67.5		
N excretion	44.30[a]	23.10[b]	3.5	0.0001
Relative	100	52.1		
Carbon				
C intake	974.43	999.07	49.62	NS
Relative	100	1.03		
C excretion	125.6	112.0	12.0	NS
Relative	100	89.2		
C retained[4]	265.84	365.34	45.67	0.06
Relative	100	1.4		

[1]Values are least square means.

[2]Effects of type of protein level (HP or VLP) on outcome parameters.

[3]Relative to HP, taken as 100%.

[4]Retained C = Digestible carbon intake – (gCO_2, d*12/44) – (gCH_4, d*12/16) – Urinary carbon.

[5]Different letters in a row denote differences at $P < 0.01$.

Table 5.8 Effect of very-reduced protein amino acid supplemented diet on gas exchange (L/d), CO_2-equivalent (kg/d) and heat production (kJ/min) of growing-finishing pigs fed ad libitum[1]

Item	High protein	Very low protein	SEM	Significance[2], P =
O_2 consumption, L/d	1.04[a3]	0.85[b]	0.04	0.02
Relative[4]	100	82		
CO_2 emission, L/d	0.86[a]	0.81[b]	0.02	0.02
Relative	100	94		
CH_4 emission, L/d	0.014	0.011	0.001	0.07
Relative	100	79		
CO_2-equvalents[5], kg/d	3.33[a]	2.98[b]	0.23	0.02
Relative	100	90		
Heat production, kJ/min	21.2[a]	18.4[b]	0.9	0.04
Relative	100	87		

[1]Both diets offered ad libitum.

[2]Effects of type of protein level (HP or VLP) on outcome parameters.

[3]Different letters in a row denote differences at $P < 0.05$.

[4]Relative to HP, taken as 100%.

[5]Calculated based on CO_2 and CH_4 measured during respiration times their GWP of 1 and 21, respectively.

Table 5.9 Suggested respiratory quotients (RQ) to be used for cattle, pigs and poultry

Species	Physiological status	Body weight (kg)	Feeding level		
			Low	Medium	High
Cattle	Dairy cows		1.0		1.2
	Breeding bulls			1.0	
	Heifers			1.0	
	Veal calves		0.8		1.0
Pigs	Weaned piglets		0.8		1.0
	Fattening pigs	20 - 50	0.98		1.05
		50 - 110	1.02		1.14
	Dry sows			1.0	
	Pregnant sows		0.75		1.1
	Lactating sows			1.0	
Poultry	Broilers		1.05		1.15
	Laying hens		0.92		0.86

Source: Adapted from van Ouwerkerk, et al. (1994).

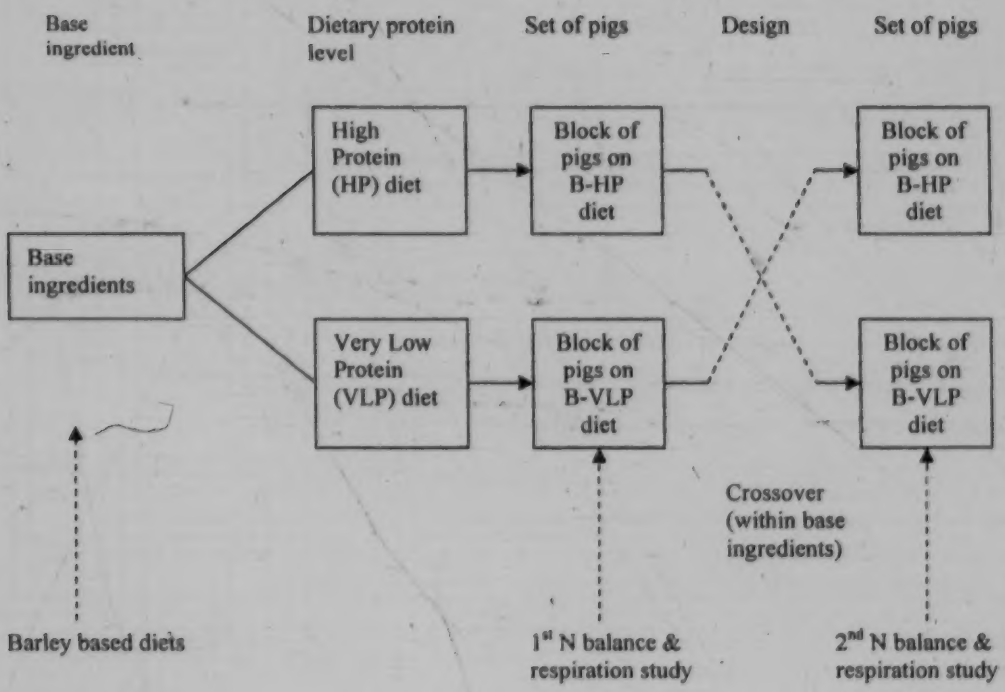
Base ingredient
Dietary protein level
Set of pigs
Design
Set of pigs
Base ingredients
High Protein (HP) diet
Very Low Protein (VLP) diet
Block of pigs on B-HP diet
Block of pigs on B-VLP diet
Block of pigs on B-HP diet
Block of pigs on B-VLP diet
Crossover (within base ingredients)
Barley based diets
1st N balance & respiration study
2nd N balance & respiration study

Figure 5.1 Schematic representation of experimental design for the study. B = Base ingredient (either corn or wheat-barley).

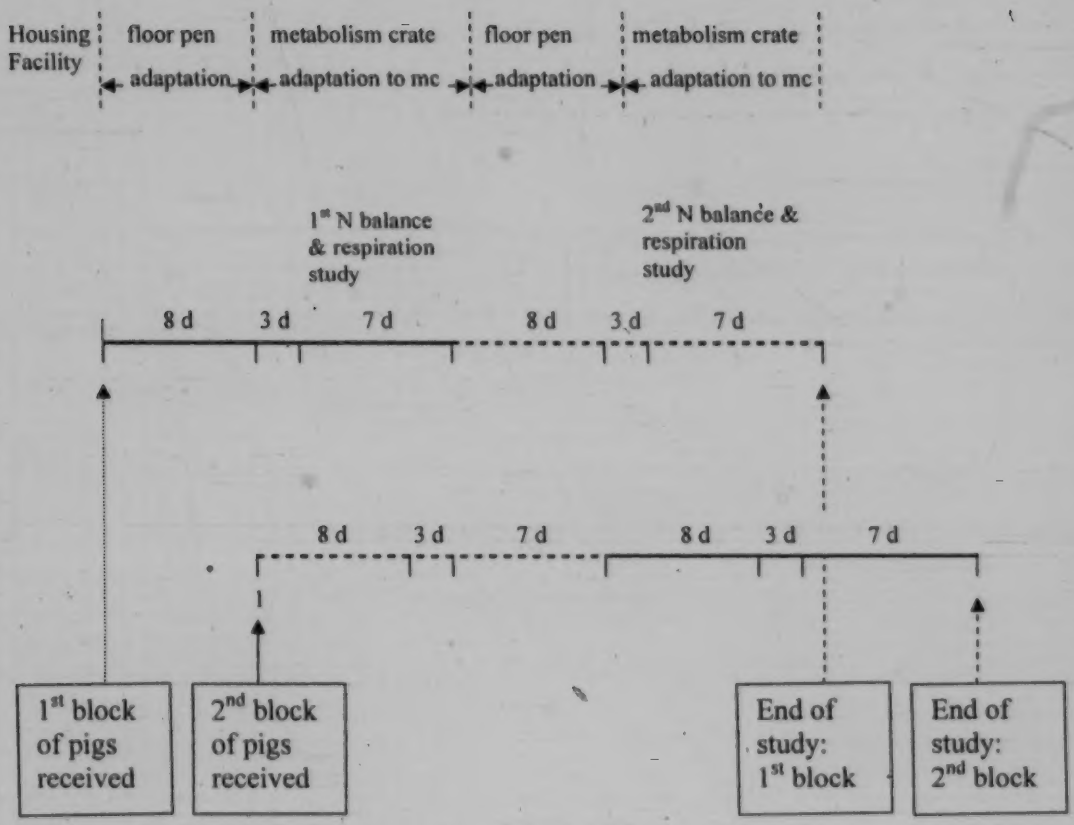

Figure 5.2 Timeline representing the housing and feeding regimen during the pre-balance, balance and respiration periods for each block of pigs.

6.0 EFFECT OF PHYTASE-XYLANASE ADDITION TO WHEAT-BASED DIETS ON PROTEIN AND ENERGY METABOLISM IN GROWING-FINISHING PIGS FED AD LIBITUM

6.1 Introduction

Aside from other goals, manipulation of swine diets aims to improve animal performance, reduce nutrient excretion and reduce cost. Lowering dietary protein while adding limiting amino acids, maintained animal performance and decreased nitrogen excretion while increasing dietary net energy (Le Bellego et al., 2001). The use of exogenous enzymes to improve the performance of poultry is not a new concept and has been studied and reviewed (Bedford, 2000; Selle et al., 2000; Acamovic, 2001; Cowieson, 2005), but much less information is available on swine.

Although the efficacy of carbohydrases, proteases and phytases in the diets of poultry has been well established, there is still a great deal of uncertainty regarding the modes of action of exogenous enzymes in both poultry and swine. Furthermore, the many interactions between enzymes and the host animal, its microflora, and dietary ingredients are not fully understood (Bedford, 2002). In the last decade, the inclusion of non-starch polysaccharide (NSP)-degrading enzymes, with predominantly xylanase activity, in wheat-based swine and poultry diets, has become routine (Bedford and Schulz,1998). As a way to reduce cost and nutrient excretion, the acceptance of microbial phytase feed enzymes has increased in response to increasing concerns over phosphorus (P) pollution in the environment. The hydrolysis of phytate by phytase increases the utilization of phytate-bound P (phytate-P) and reduces P excretion, but some research suggests that phytase also improves protein and energy utilization (Ravindran et al., 1999). Phytase addition to pig diets reduced phosphorus excretion (Oryschak et al., 2002; Adeola and Sands, 2003), and may improve pig performance (Kim et al., 2005). Phytase supplementation to diets limiting in P improved ADG and G:F in young pigs (Adeola et al., 2004). However, the lack of increased ADG might be expected in experiments where P was not limiting in the diets, because excess dietary P does not increase ADG (Ekpe et al., 2002). Similarly, xylanase addition to wheat-based diets may also improve nutrient

digestibility and pig growth (Kim et al., 2005). Oryschak et al. (2002) reported that a reduction in dietary DE by phytase addition to wheat-based diets was nullified by xylanase addition. Whereas, Kies et al. (2005) reported marginal increase in energy retention in pigs fed phytase supplemented diets with the same phosphorus content in the diets. Qu et al. (2005) reported that limiting dietary phosphorus may affect enzymes of energy metabolism and recommended that phytase effects on energy metabolism should be investigated in diets with lowered phosphorus.

Wheat successfully competes with corn as feed ingredient for swine and poultry. It possesses some advantages over corn, such as superior pelleting characteristics and higher content of crude protein and lysine (Crouch et al., 1997). High content of arabinoxylans in the endosperm cell wall of wheat and its by-products (e.g., wheat middlings and wheat millrun), however, impairs nutrient availability, decreases metabolizable energy and, consequently, lowers performance of swine and poultry fed on wheat-based diets (Marquardt and Han, 1997). Although wheat by-products generally have a greater content of NSP, CP and minerals than the parent wheat (Slominski et al., 2004), nutrients such as AA are digested to a lesser extent than in the parent grain (Sauer et al., 1977). Pigs do not digest feedstuffs with a high NSP content well (Barrera et al., 2004); therefore the DE content of most grain by-products is low (NRC, 1998). In wheat by-products (e.g., middlings), P is partly bound as phytate-P (Garcia-Estepa et al., 1999). Pigs do not produce endogenous phytase (Golovan et al., 2001); therefore are not efficient in hydrolyzing phytate (Pointillart et al., 1984) resulting in a reduced digestibility of P in grains and their by-products (NRC, 1998). Some attempts have been made to alleviate these disadvantages by means of diet supplementation with a suitable microbial endoxylanase.

The application of xylanase in poultry nutrition is well established (Crouch et al., 1997; Marquardt and Han, 1997), but there are few experiments in swine nutrition (Feoli et al., 2005). Possible interactions between phytase and xylanase following their simultaneous inclusion in wheat-based broiler diets have attracted interest in recent years (Ravindran et al., 1999). Despite the increasing likelihood of phytase and xylanase being used

simultaneously in swine and poultry diets, published reports on their combined application in poultry (Cowieson et al., 2006; Zyla et al., 1999a, b; Newkirk and Classen, 1993; Adrizal et al., 1996) and swine (Olukosi et al., 2005; Woyengo et al., 2005; Atakora et al., 2006a, b; Nortey et al., 2006) are limited. One area that has received relatively little attention in the literature is the use of combinations of enzymes such as xylanases and β-glucanases, and phytases to improve nutrient retention and performance. Logic would suggest that if the use of each enzyme can improve body weight gain and feed conversion ratio in poultry compared with birds that have been fed a diet containing no enzyme, the use of two or more enzymes might improve the scale and consistency of the response. However, the reports in the literature are inconsistent, and include results showing that the combination of phytase and xylanase are: antagonistic (Naveed et al., 1999; Saleh et al., 2004), subadditive (Zyla et al., 2000; Wu et al., 2004; Kim et al., 2005; Leslie et al., 2005), additive (Zyla et al., 1996; Mulyantini et al., 2005) and synergistic (Ravindran et al., 1999; Oryschak et al., 2002). Therefore, it is critical that more information is generated and more metabolic techniques, such as whole body calorimetry, be applied to elucidate phytase-xylanase effects. In the present experiment it was hypothesized that phytase and xylanase inclusion in growing-finishing pigs' diet would improve carbohydrate and protein utilization and reduce carbon dioxide and methane emission. The aim of the study was to examine the interaction among dietary protein (CP) and phosphorus reduction with phytase or xylanase individually, or in combination, on performance, total tract nutrient digestibility and energy metabolism measured by indirect calorimetry in growing-finishing pigs fed wheat-based diets *ad libitum*.

6.2 Materials and Methods

The experimental proposal and procedures for care and treatment of the growing-finishing gilts were reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Policy and Welfare Committee (FAPWC) of the University of Alberta to ensure adherence to the Canadian Council of Animal Care (CCAC, 1993) guidelines.

6.2.1 Enzymes

The following enzymes (manufactured and supplied by Danisco Animal Nutrition, Malborough, UK) were used: phytase (Phyzyme XP5000; activity, 500 FTU/kg) and xylanase (Porzyme 9300; activity, 4000 XU/kg) (Table 6.1). The xylanase was endo-1, 4-b-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Malborough, UK) and the phytase was 6-phytase (EC 3.3.26; Phyzyme XP; Danisco Animal Nutrition, Malborough, UK). Phytase activity (one unit of phytase (FTU) is defined as the quantity of phytase required to release 1 μmole of inorganic phosphorus per min from 0.00015 mole/l sodium phytate at pH 5.5 at 37°C). Xylanase activity (one unit of xylanase (XU) is defined as the quantity of xylanase required to release 1 μmole of xylose in one minute at pH 5.3 at 50°C)) and β-glucanase activity (one unit of β-glucanase (BGU) is defined as the amount of enzyme that liberates 1 μmole of reducing sugars (expressed as glucose) in one minute at pH 5.0 at 30°C)). Xylanase and phytase were included at 153 and 100 g/metric ton of finished feed, respectively, (Table 6.1) as recommended by the manufacturer and supplier (Danisco Animal Nutrition, Malborough, UK). Granular formulations of the enzymes were used.

6.2.2 Experimental Design

Seventy-two Genex F2 (Large White x Landrace) gilts (58.0 ± 0.7 kg, initial body weight), received one of six diets in twelve replicates of six pigs, for a period of 21 d. The pigs were adapted to individual housing and diets for 7 d. This was followed by 3 d adaptation to adjustable metabolism crates (183 cm x 61 cm x 88 cm; and 62 cm above concrete floor) with rubber-coated wire mesh floor and a 7 d nitrogen and energy balance period. Energy metabolism was then measured by indirect calorimetry. The effects of protein and phosphorus reductions, the inclusion rates of phytase (0 or 500 FTU/kg of feed), and xylanase (0 or 4000 XU/kg of feed) were tested in a 2 x 2 (+2) factorial arrangement of treatments in a randomized complete block (RCB) design, involving six wheat-based diets.

6.2.3 Diet Composition

Six iso-energetic diets, based on 76 – 86% of wheat and 10% wheat middlings, were formulated to achieve equal standardized ileal digestible contents amino acids (lysine, methionine, tryptophan and threonine), minerals (except phosphorus) and vitamins (Table 6.1) to meet or exceed requirement (NRC, 1998) by fifteen percent. The control diet (Diet Con = high protein-adequate phosphorus) contained protein-bound amino acids only, supplied by soybean meal. The other five diets were formulated without soybean meal to be low-protein (LP) diets; L-lysine HCl and L-threonine (Degussa, AG, Germany) were supplemented to meet requirements according to NRC (1998). The low protein diet 'LP+' (low-protein-adequate phosphorus) contained dicalcium phosphate to elevate total phosphorus contents to the recommended level. The 'LP-'diet (low-protein without supplemental phosphorus) had no dicalcium phosphate added, but calcium was adjusted to the level in LP+ with limestone (calcium carbonate). The remaining diets were identical to LP-, except for the addition of 500 units of phytase (Diet Phy), 4000 units of xylanase (Diet Xyl) or 500 units of phytase plus 4000 units of xylanase (Diet PhyXyl). All diets were fed without or with enzyme (individually or in combination to provide a guaranteed minimum of 500 FTU/kg and 4000 XU/kg of phytase and xylanase, respectively) shown in Table 6.1.

6.2.4 Housing and Feeding

The growing-finishing Genex F2 (Large White x Landrace) gilts were received from the closed population of group-housed pigs at the Swine Research and Technology Centre and individually housed at the Metabolic Unit, University of Alberta research station, in twelve replicates of six pigs. Feed and water were offered ad libitum. The animals were adapted to individual floor housing and diet for at least 7 d prior to the nitrogen balance study. Feed not eaten or spilled was collected, dried, weighed and deducted from the daily feed allowance to calculate net feed intake. Fresh feed was offered twice daily, except for the respiration studies during which half the previous day's allowance was offered in eight equal hourly meals, two hours prior to the commencement of respiration studies to facilitate feed consumption, reduce wastage in the chambers and reduce variation due to individual differences in feeding frequency. This made it made it

possible to monitor the activities (feeding and resting, and gut motility patterns) and relate these to calorimetry data.

6.2.5 Body Weight Measurements

Body weights of the pigs were measured weekly at the beginning, end, and before and after nitrogen balance and respiration studies to the nearest 100 g on a scale (Accurate Scale Industries, Ltd., Edmonton, AB) with a digital readout (DF 2000, Massload Technologies, Saskatoon, SK).

6.2.6 Nitrogen and Energy Balance

During the diet adaptation period, the pigs were repeatedly confined in respiration chambers (234.5 cm x 106 cm x 86 cm) to acclimatize them to the respiration boxes prior to start of respiration studies. After an additional 3 d adaptation to adjustable metabolism crates (183 cm x 61 cm x 88 cm; and 62 cm above concrete floor) with a rubber-coated wire mesh floor, a 7 d nitrogen balance period with quantitative collection of feces and urine was used to measure fecal digestibilities, nitrogen and carbon retention in individual pigs. Fecal collections were made as frequently as animals defecated and stored in covered plastic buckets (collected feces were stored in a cooler (4°C) and frozen (-20°C) at the end of each collection day. At the end of a balance period the pooled 7 d fecal collection was weighed, thawed and homogenized. Sub-samples (360 ± 10 g) of homogenized feces were placed in aluminum pans (in duplicate) covered with polythene bags and stored at -20°C prior to freeze drying. Freeze-dried samples were ground with coffee grinder and stored in sealed plastic containers until chemical analyses. Daily urine collections were done under 20 ml concentrated H_2SO_4 to prevent volatilization of urine-N as NH_3, and also prevent bacterial growth, in plastic buckets with lids placed under a V-shaped stainless steel collection tray covered with 0.5 mm screen, thus preventing fecal materials from contaminating the urine collections. The 24 h daily urine collections started at 1000 to 1000 h the following day on each day during the balance period. At the end of each 24 h collection, the urine was weighed, thoroughly stirred and sub-sampled. Five percent aliquot samples (w/w) from daily collections were pooled into brown bottles stored in the cooler till the end of balance collections. At the end of the 7 d the pooled

aliquots of urine were weighed, thoroughly homogenized, and sub-sampled. Sub-sampled urine collections (100 ml) were stored at -20°C in covered plastic containers until thawed for chemical analyses.

6.2.7 Respiration Measurements

Gas exchange was determined over 24 h immediately after the nitrogen balance period using an open circuit indirect calorimetry. Respiration measurements were performed in an open-circuit indirect calorimeter. The respiration chambers (234.5 cm x 86 cm x 106 cm) consisted of commercial farrowing crates enclosed in plexiglass boxes, equipped with a feeder and low pressure nipple drinker, and a cooler to maintain temperature in the thermoneutral zone. Air was drawn through boxes, via an inlet at the rear and an outlet above the feed trough, at rates of approximately 240 to 250 L/min. Air flow was measured after passing drawn air through a cold water condenser, to remove water vapor, with temperature-compensated commercial air meters (Model 1023, Canadian Meter Corp., Cambridge, Canada). A sample of air was drawn with small air pump (Gast Model 0531, Gast Mfg. Corp., Benton Harbour, MI) and delivered to a fuel-cell type O_2 (Taylor-Servomex, Crowborough, UK) and a non-dispersive near infra-red CO_2 analyzers (Beckman LB2, Beckman, Irvine, CA), and CH_4 analyzers (Qubit Systems, Kingston, ON, Canada). Air flow to the analyzers was regulated to 0.5 L/min by ball-type flow meters (Scienceware Size 2, Fisher Scientific, Mississauga, Canada). The analog output (mV) of the analyzers was converted to digital data by an analog-digital converter (Data grabber, Data Electronics, Australia) and recorded by a computer. Data acquisition was set for maximum rate (four readings per second) and the average gas concentration for each minute was recorded. For each study, the O_2 and CO_2, and CH_4 (Qubit Systems, Kingston, ON, Canada) analyzers were calibrated for zero and gain readings with either pure N_2 (zero) or calibration gas (1% CO_2, 20% O_2, and 79% N_2). The span gas was composed of 19.44% O_2, 1.53% CO_2, 105 ppm CH_4 and balance N_2. Measurements of these gases at steady state and of room air were recorded before and after the study period. During the respiration measurements (1 h equilibration plus 24 h study) the pigs were confined in the respiration chamber. Expired air was analyzed continuously for O_2, CO_2 and CH_4 contents during the 24 h. During respiration the pigs received half of their

daily allowance in eight hourly meals, plus the other half (in bulk) after the hourly feedings.

6.2.8 Chemical Analyses

Feed, fecal and urine samples were stored in a -20°C freezer prior to chemical analyses. Samples of each diet were taken weekly, and pooled within diet type. Pooled feed samples were analyzed for amino acids by Degussa AG, (Hanau, Germany) using ion exchange chromatography (Llames and Fontaine, 1994). Tryptophan was analyzed by fluorescence detection HPLC after alkaline hydrolysis with barium hydroxide octahydrate at 110°C for 20 h (Fontaine et al., 1998). Gross energy of feed and fecal samples was determined using an adiabatic bomb calorimeter (Leco AC-300, LECO Corp., St. Joseph, MI). Feed, fecal and urine N content were determined according to macro-Kjeldahl (AOAC, 1998). Proximate analyses for dry matter, ash, ether extract (Göldfisch extraction apparatus, Labconco Corp., Kansas City, MO), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Ankom Fiber Technique, Goering and van Soest, 1970) were determined on feed and fecal samples. Total carbon content of feed and freeze-dried fecal samples ground in a MM2 Retsch/Birkmann mixer mill (Brinkmann Instruments Inc., Westbury, NY), were analyzed using a NA 1500 Carlo-Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ). Prior to proximate analyses, feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a 1.0 mm screen.

6.2.9 Calculations

Nitrogen retention (RN) was calculated as the difference between N intake and N losses in feces and urine, excluding N losses in gas (water vapour and ammonia) originating from feces since condensed water and extracted air were not analyzed for N. The DE and ME values of the diets were calculated according to methods described by Noblet et al. (1987). For the ME calculation, the average CH_4 emission was applied to all pigs.

Heat production (HE) was calculated based on gas exchanges (indirect calorimetry) according to the formula of Brouwer (1965), including CH_4 emission and urinary N

excretion (U_N). Retained energy (RE) was calculated as the difference between ME intake and HE over the measurement period. RE as protein (RPE) was calculated from the N balance, and energy retained as fat (RFE) was calculated as the difference between RE and RPE (Noblet et al., 1987). Data related to the energy balance were expressed relative to BW raised to the power of 0.75.

During the nitrogen balance period, whole tract digestibility of nitrogen, phosphorus, calcium, ether extract, NDF, ADF and gross energy were determined. Fat and energy retention were calculated according to C-N balance technique, assuming 23.8 kJ/g deposited protein, and 39.6 kJ/g deposited lipid (Noblet et al., 1987; Moehn and Susenbeth, 1995). The amount of digested nutrients and energy, and nitrogen and energy excretion with urine, was measured. Deposited protein (DP) and fat (DF) and carbohydrate (DCHO) expressed in energy units were calculated from the 7d balance experiments as:

DP, kJ = diN, g x 6.25 x 23.86, kJ/g, where diN (g) = digested nitrogen
DF, kJ = diF, g x 39.76, kJ/g, where diF (g) = digested fat and oils
DCHO, kJ = DE, kJ – DP, kJ – DF, kJ, where DE = digestible energy

Maintenance net energy (NEm) was taken as the intercept of the regression of heat production (HE) on feed intake (per $kg^{0.75}$ body weight, Noblet et al., 1987). Dietary net energy (NE) was calculated as retained energy (RE) plus maintenance net energy (NEm). Using the modified method of Goering and van Soest (1970), hemicellulose was calculated as the difference between NDF and ADF.

Factors and constants taken from Brouwer (1965) and Wenk et al. (2001) were used in all calculations. Heat production (HE) was based on the measurements of gas exchange and 7 d determination of nitrogen excreted in urine (U_N) and was calculated as:

$$HE\ (kJ) = 16.18*V_{O2} + 5.02*V_{CO2} - 5.99*U_N - 2.17*V_{CH4}$$ (Brouwer, 1965).

Retained (or mobilized) energy in protein (RPE) was calculated from the nitrogen balances and gas exchange measurements as:

RPE, kJ = (diN – U_N) x 6.25 x 23.86, kJ/g

Retained (or mobilized) energy in fat (RFE) was calculated:

RFE, kJ = ME – HE – RPE, where ME = metabolizable energy (kJ); HE = heat production (kJ); RPE = retained energy in protein (kJ).

For comparison, the RPE and RFE were also calculated from carbon (C) balances as:

C in CO_2 emitted, g = CO_2 emission, litres x 0.536, g/L

C in RP, g = RP, g x 0.52, where 0.52 is average C content in protein

C balance, g = C intake, g – (C in feces, g + C in urine, g + C in CO_2, g)

RFE, kJ = C in RF, g/0.767 x 39.76, kJ/g, where 0.767 is an average C content in fat.

Carbon retention (RC) was calculated as:

RC = C intake – (fecal C + urinary C) – (12/44*CO_2 d^{-1}) – (12/16*CH_4 d^{-1}).

The C content of gases and urine was taken as: 12 g/mol for CO_2 and CH_4, and as 90% of the N content of urine (Schiemann et al., 1971). The carbohydrate content of the animals was assumed to remain constant during the respiration period.

Fat retention (RF) was calculated as:

$C_{retention}$ minus $C_{retained}$ in protein (52%) divided by the C content in body fat (76.7% as reported by Schiemann et al. (1971)).

Metabolizable energy (ME) intake was calculated as:

ME, kJ = DE – CH_4E – UE, where DE = digestible energy (kJ/g); CH_4E = energy in CH_4 (39.56 kJ/L; Schiemann et al., 1971); UE = energy in urine (UE, kJ/g = 0.333 * % C + 0.093 * % N, Hoffmann and Klein, 1980).

RE was taken as energy retained as protein and fat (22.6 kJ/g and 39.6 kJ/g respectively; Böhme and Gädecken, 1980).

Respiratory quotient (RQ), based on the measurements of gas exchange and 7 d determination of nitrogen excreted in urine (U_N) was calculated as: $RQ = V_{CO2}/ V_{O2}$. The RQs were calculated to determine the energy substrate utilized by the growing-finishing pigs during the study period. CO_2-equivalents, from the growing-finishing pigs, were calculated using factors of the global warming potential (GWP): 1, 21 and 310 for CO_2, CH_4 and N_2O respectively, on a molar basis (Grubb et al., 1999; IPCC, 1997). The conversion of nutrients in manure to greenhouse gases, is not well established, therefore, the range in CO_2-equivalents from manure were calculated using the range in estimated conversion rates of 5 or 30% of the N in manure to N_2O (Béline et al., 1999), and 3 or 20% of C in manure to CH_4 (Martinez et al., 1999). The contents of N and C in manure were taken as the excreted N and C by the pigs. Total excretion values (TN and TC respectively) per pig were calculated as the sum of fecal and urinary N and C excretion.

6.2.10 Statistical Analysis

The data were subjected to mixed model procedure (SAS, 2005), to assess the impact of the factors 'protein level', phosphorus level', 'phytase addition' and 'xylanase addition' on dependent variables. The model included the phytase x xylanase interaction, body weight and feed intake. 'Block', 'pen' and 'respiration chamber', as well as interactions between random and main variables were tested. The effects of protein and phosphorus levels were compared to the control diet (Con) and contrast statements were used for separation of dietary least square means. The total number of observations was 72.

To determine enzyme effects, the treatments phytase, xylanase and phytase x xylanase interaction were analyzed as 2 x 2 factorial arrangement of treatments (plus the two diets:

Con and LP+) in a randomized complete block (RCB) design (Milliken and Johnson, 1984). The statistical model included the following effects: phytase (none and some), and xylanase (none and some) and their interaction term, according to the design below:

Two-way treatment structure for phytase and xylanase addition to a base diet[1]

	Xylanase	
Phytase	*None*	*Some*
None	Base	Base + Xylanase
Some	Base + Phytase	Base + Phytase + Xylanase

[1]Base = Low protein low phoshorus (LP-) diet; Base + Xylanase = Xyl diet; Base + Phytase = Phy diet; Base + Phytase + Xylanase = PhyXyl diet as in Tables 6.1 – 6.11.

The number of observation per treatment was 12. The initial weight of the pig was used as covariate. Results for the dependent variables are presented as least square means. Individual pig was considered as the experimental unit. The 'pdiff" option (SAS, 2005), was used for means separation. Significance was declared at $P < 0.05$ and $P < 0.1$ was regarded as a trend.

6.3 Results

The calculated and analyzed compositions of the experimental diets (Tables 6.1 and 6.2) were in agreement with the aim of the experiment with regards to the dietary protein (CP) and phosphorus levels in all diets. The mean lysine level was 0.69% (range: 0.66 to 0.74%). The calcium levels (mean: 0.71%, range: 0.64 to 0.77%). The Ca:total P ratio averaged 1.48 (range: 1.30 to 1.70) was lowest (1.30) in diet Phy, thereby reflecting that phytase effect was tested in a lowered phosphorus diet compared to the levels in the Con (positive control) and LP+ (negative control) diets. The analyzed P content averaged 0.48% (range, 0.44 to 0.54%) was, however, 4.1 to 14.6% higher than formulated total P content (Tables 6.1 and 6.2). Diet PhyXyl was greatest in NDF (overall range, 16.8 to 19.9% DM) and diet LP+ was greatest in ADF (overall range, 4.2 to 5.0% DM) (Table 6.2).

The chemical composition of feces and urine from pigs fed the six dietary treatments, including ether extracts, crude ash, NDF, ADF, carbon, phosphorus, calcium, nitrogen and gross energy were determined (Table 6.3) and used for calculations of digestibilities and retention of energy and nutrients and heat production.

6.3.1 Dietary Protein Reduction

The dietary protein reduction (Con vs. LP+) did not affect ($P > 0.10$) any of the growth performance traits (i.e., final weight, average daily feed intake (ADFI), average daily gain (ADG), and Gain:Feed) (Table 6.4). The dietary protein reduction lowered N intake (13.2 g/d; $P < 0.0001$) and urine nitrogen excretion (13.6 g/d; $P < 0.0001$), however, animal performance was maintained (Table 6.5). This may be due to a good balance of amino acids and reduced expenditure of energy for excretion of excess protein in the reduced protein diet (LP+) compared to the Con diet. Fecal nitrogen reduced (1.8 g/d; $P = 0.0002$) with the dietary protein reduction (Table 6.5). The dietary protein reduction improved ($P = 0.001$) percent N retention, but did not affect (P = 0.49) retained N in grams per day (Table 6.5).

The dietary protein reduction (Con vs. LP+) lowered feces carbon (13.0 g/d; $P = 0.04$) and urine carbon (12.3 g/d; $P < 0.0001$). However, the protein reduction did not affect ($P > 0.10$) intake of carbon, CO_2 emission, CH_4 emission, retained carbon (g/d), and percent carbon retention (Table 6.6). The energy metabolism parameters: ME intake (MJ/d), HE (MJ/d), RE (MJ/d), NE (MJ/kg), RE:ME ratio and RQ were not affected ($P > 0.10$; Table 6.7) by dietary protein reduction.

However, the dietary protein reduction increased measured DE (0.2 MJ/kg; $P = 0.05$), and increased measured ME (0.5 MJ/kg; $P = 0.0002$). The dietary protein reduction improved the digestibility of GE (1.1 pu; $P = 0.04$), nitrogen (1.1 pu; $P = 0.03$), calcium (4.6 pu; $P = 0.02$), phosphorus (8.3 pu; $P = 0.0002$), crude fat (3.6 pu; $P = 0.001$), NDF (4.0 pu; $P = 0.005$), and ADF (10.0 pu; $P = 0.0001$) compared to diet Con. The impact of the dietary reduction on carbon digestibility was a tendency ($P = 0.07$) (Table 6.8).

Considering the partitioning of dietary energy in the growing-finishing pigs, in this experiment, the protein reduction (Con vs. LP+) lowered fecal energy (0.5 MJ/kg; $P = 0.04$), and lowered urinary energy (0.6 MJ/kg; $P < 0.0001$). As indicated earlier (Table 6.8) the dietary protein reduction increased measured DE ($P = 0.05$) and measured ME ($P = 0.0002$). However, it did not influence CH4E (MJ/kg), NE (MJ/kg), and HI (MJ/kg) ($P > 0.10$; Table 6.9).

In this experiment, energy losses in feces (3.1 pu; $P = 0.03$) and urine (3.5 pu; $P < 0.0001$) to relative to energy intake were lowered. The reduction, however, did not affect ($P = 0.11$) energy losses in CH_4 (Table 6.10). Whilst the protein reduction improved ($P = 0.001$) the efficiency of nitrogen utilization (kn), it did not influence (P = 0.62) efficiency of protein and fat utilization (kpf) and also when expressed as the RE:ME ratio (Table 6.10). In addition, the dietary protein reduction affected the ratios of ME:GE ($P = 0.0002$), ME:DE ($P < 0.0001$), and UE:DE ($P < 0.0001$). The effect of the protein reduction on CH_4E:DE ratio was a tendency ($P = 0.10$). The dietary protein reduction did not affect the ratios of DE:GE, NE:ME, HE:ME and RE:ME ($P > 0.30$; Table 6.10).

The dietary protein reduction did not affect all parameters of gas exchange (O_2 consumption, CO_2 emission and CH_4 emission), greenhouse gas emission expressed as CO2-equivalent (based on CO_2 and CH_4 individually), RQ, HE and HI ($P > 0.10$; Table 6.11). The effect of the protein reduction on total CO_2-equivalent (CO_2 plus CH_4) was a tendency ($P = 0.08$; Table 6.11).

6.3.2 Phosphorus Reduction

The phosphorus reduction (LP+ vs. LP-) did not influence many of the response variables in this experiment. The reduction in phosphorus level did not affect ($P > 0.20$) any of the growth performance parameters, similar in pattern to the dietary protein reduction (Table 6.4). From the nitrogen balance studies, phosphorus reduction did not influence ($P > 0.40$) any of the N balance parameters (Table 6.5). In a similar pattern reduction in phosphorus level did not affect any of the carbon balance parameters ($P > 0.50$; Table 6.6). Also, phosphorus reduction did not influence any of the parameters of energy

metabolism – ME intake (MJ/d), HE (MJ/d), RE (MJ/d), NE (MJ/kg), RE:ME ratio and RQ ($P > 0.60$; Table 6.7).

Similar to the above, phosphorus reduction did not affect both measured DE and ME ($P > 0.50$; Table 6.8). Also, the effect of the phosphorus reduction did not influence the digestibility of GE, nitrogen, carbon, calcium and phosphorus ($P > 0.10$; Table 6.8). However, phosphorus reduction showed tendency to affect the digestibility of crude fat ($P = 0.08$; Table 6.8). In this experiment, the digestibility of NDF (3.8 pu; $P = 0.05$) and ADF (9.6 pu; $P = 0.0002$) was poorer in the pigs fed the LP- compared to LP+ diet (Table 6.8). With respect to partitioning of dietary energy in the pigs, in this experiment, phosphorus reduction did not affect ($P > 0.50$) any of the parameters (Table 6.8).

The effect of phosphorus reduction (LP+ vs. LP-) on relative energy losses (in feces, urine and CH_4) to energy intake was not significant ($P > 0.40$; Table 6.10). Omission of phosphorus did not influence ($P > 0.80$) efficiency of utilization of nutrients (kn and kpf) (Table 6.10). Whilst the reduction in phosphorus level did not affect most of the energy ratios – ME:DE, UE:DE, CH_4E:DE, NE:ME, HE:ME and RE:ME ($P > 0.60$), the reduction improved the DE:GE ratio ($P = 0.04$) and ME:GE ratio ($P = 0.04$) (Table 6.10). The phosphorus reduction did not influence the parameters of gas exchange (O_2 consumption, CO_2 emission, and CH_4 emission), greenhouse gas emission (CO_2-equivalent), RQ, HE and HI ($P > 0.10$; Table 6.11).

6.3.3 Enzyme Supplementation

The effects of enzyme supplementation to the basal LP- diet are considered with respect to: (i) phytase addition, (ii) phytase-xylanase interaction and (iii) xylanase addition.

6.3.3.1 Phytase Addition

Phytase addition to the LP- diet did not affect ($P > 0.20$) any of the growth performance traits (Table 6.4). Similarly, phytase did not influence any of the parameters of nitrogen balance ($P > 0.40$; Table 6.5) and carbon balance ($P > 0.10$; Table 6.6). Similar to the

above pattern, phytase addition to the LP- diet did not affect ($P > 0.20$) the parameters of energy metabolism (Table 6.7).

Whilst phytase addition to the LP- diet did not affect measured DE and measured ME ($P > 0.40$) and also did not influence the digestibility of GE, nitrogen, carbon, calcium and crude fat ($P > 0.30$), it affected the digestibility of phosphorus ($P = 0.001$), NDF ($P = 0.001$) and ADF ($P = 0.008$) (Table 6.8). Phytase supplementation did not influence the partitioning of dietary energy in the pigs ($P > 0.10$; Table 6.9). In a similar pattern, phytase supplementation to the LP- diet did not influence parameters of energy losses (in feces, urine and CH_4) relative to energy intake ($P > 0.10$), efficiency of nutrient utilization ($P > 0.30$), and the ratios of ME:GE, ME:DE, UE:DE, CH4E:DE, NE:ME, HE:ME, and RE:ME ($P > 0.10$; Table 6.10). However, the phytase supplementation improved ($P = 0.01$) the DE:GE ratio (Table 6.10). Phytase addition did not influence the parameters of gas exchange (O_2 consumption, CO_2 emission, and CH_4 emission), greenhouse gas emission (CO_2-equivalent), RQ, HE and HI ($P > 0.10$; Table 6.11).

6.3.3.2 Phytase-Xylanase Interaction

The phytase-xylanase interaction affected ($P = 0.05$) final weight (Table 6.4). Thus, phytase and xylanase acted together to influence the final weight of the pigs. Whilst the interaction did not influence ($P > 0.20$) feed intake and gain:feed, its effect on daily gain was a trend ($P = 0.06$) in this experiment (Table 6.4). The interaction of phytase and xylanase did not influence any of the parameters of nitrogen balance ($P > 0.40$; Table 6.5). Similarly in this experiment, phytase-xylanase interaction did not affect ($P > 0.50$) the carbon balance parameters (Table 6.6).

The phytase-xylanase interaction did not influence ($P > 0.20$) the parameters of energy metabolism of the growing-finishing pigs in this experiment (Table 6.7). The interaction of the enzymes affected measured DE ($P = 0.05$) and measured ME ($P = 0.03$), but did not affect ($P > 0.10$) the digestibility of GE and nutrients (Table 6.8). Also, the phytase-xylanase interaction did not influence ($P > 0.60$) the partitioning of dietary energy (Table 6.9).

Considering the energy losses in feces, urine and CH_4 relative to energy intake, the phytase-xylanase interaction did not affect ($P > 0.50$) any of the parameters (Table 6.10). Similarly, the interaction did not influence the efficiency of nutrient utilization ($P > 0.20$) and the energy ratios ($P > 0.10$) (Table 6.10). Also, the parameters of gas exchange, greenhouse gas emission (CO_2-equivalent), RQ, HE and HI were not affected by the phytase-xylanase interaction ($P > 0.70$; Table 6.11).

6.3.3.3 Xylanase Addition

The addition of xylanase to the LP- diet (LP- vs. Xyl) did not influence ($P > 0.20$) any of the growth performance traits (Table 6.4). Also, xylanase supplementation to LP- did not affect nitrogen balance ($P > 0.10$; Table 6.5). Generally, the xylanase addition did not influence ($P > 0.10$) the parameters of carbon balance; however, it showed tendency to influence CH_4 emission (Table 6.6). Numerically, the CH_4 emission value from the pigs fed the xylanase-added diet (Xyl) was similar to that pigs fed the positive control (Con) diet. Generally, the pigs fed the enzyme-added and LP- diets emitted lower CH_4 (g/d) than the Con diet (Table 6.6).

In this experiment, energy metabolism parameters including ME intake (MJ/d), HE (MJ/d), RE (MJ/d), RE:ME intake ratio, NE (MJ/kg) and RQ were not influenced ($P > 0.10$) by xylanase supplementation to LP- diet (Table 6.7). Xylanase addition to the LP- diet increased measured DE (0.3 MJ/kg; $P = 0.02$), but did not affect ($P = 0.60$) measured ME (MJ/kg) (Table 6.8). The addition of xylanase to the LP- diet improved the digestibility of GE (1.0 pu; $P = 0.04$), nitrogen (1.1 pu; $P = 0.05$), carbon (1.0 pu; $P = 0.05$), crude fat (1.5 pu; $P = 0.03$), NDF (2.3 pu; $P = 0.04$), and ADF (6.0 pu; $P = 0.03$) (Table 6.8). As expected, xylanase supplementation did not influence calcium and phosphorus digestibility ($P > 0.30$; Table 6.8).

With respect to partitioning of dietary energy, xylanase addition to LP- diet affected ($P = 0.02$) measured DE by 0.3 MJ/kg (Tables 6.8 and 6.9), but its effect on CH_4 was trend ($P = 0.09$), similar to its effect on CH_4 emission (g/d) as in Table 6.6. Xylanase addition did

not affect FE, ME, UE, NE and HI ($P > 0.10$; Table 6.9). In a similar pattern, relative energy losses of energy intake (%) in feces and urine were not affected ($P > 0.20$) by xylanase supplementation (Table 6.10). The effect of xylanase supplementation on CH_4 emission was a trend ($P = 0.06$; Table 6.10), comparable to the effect on CH_4E:DE ratio ($P = 0.10$; Table 6.9). Xylanase supplementation did not affect ($P > 0.20$) efficiency of nutrient utilization (kn and kpf); however, it improved ($P = 0.001$) the DE:GE ratio (Table 6.10). The rest of the energy ratios – ME:GE, ME:DE, UE:DE, NE:ME, HE:ME and RE:ME were not affected by xylanase supplementation to LP- diet in this experiment (Table 6.10). However, the effect of xylanase inclusion on CH_4E:DE was a tendency ($P = 0.10$; Table 6.10). Similarly, showed tendency to influence CH_4 emission ($P = 0.09$), CO_2-equivalent based on CH_4 emission alone (P = 0.09) and total CO_2-equivalent based on CO_2 plus CH_4 emissions ($P = 0.08$) (Table 6.11). Xylanase supplementation did not influence gas exchange (O_2 consumption and CO_2 emission), RQ, HE and HI ($P > 0.10$; Table 6.11).

6.4 Discussion

The aim of the study was to examine the effect of dietary protein (CP) and phosphorus reduction, phytase or xylanase addition, individually and in combination, and phytase-xylanase interaction on growth performance, total tract nutrient digestibility and energy metabolism in growing-finishing pigs fed ad libitum on a wheat-based diet. This experiment was primarily designed to study energy metabolism and nutrient balance. The levels of dietary factors (protein and phosphorus levels and individual or combined enzyme levels) allowed the dietary effects to be tested.

In this study, the reduction of dietary CP (Con vs. LP+) in diets for growing-finishing pigs did not affect growth performance (Table 6.4).

The reduction of dietary CP lowered fecal and urinary carbon (g/d), but increased retained carbon (%). The CP reduction did not affect CO_2 and CH_4 emission (Table 6.6). The CP reduction did not affect energy metabolism (Table 6.7), but increased DE and ME intakes, and digestibility of GE and nutrients (Table 6.8). In this experiment lowered

dietary CP reduced energy partitioning in feces and urine, but increased DE and ME, MJ/kg (Table 6.9). The lowered dietary CP reduced energy losses via feces and urine, increased percent N retention (kn), increased energy utilization ratios – ME:GE and ME:DE, but lowered UE:DE ratio. The ratio CH_4:DE showed tendency to decrease (Table 6.10). The reduction in dietary CP increased total GHG (Table 6.11).

Omission of phosphorus (LP+ vs. LP-) reduced digestibility of NDF and ADF (Table 6.8) and reduced energy utilization ratios – DE:GE and ME:GE (Table 6.10). Phytase supplementation to the negative control diet (low-protein, low-phosphorus, LP-) improved digestibility of NDF and ADF (Table 6.8). Xylanase supplementation to same diet (negative control) improved DE intake, increased digestibility of GE, carbon, NDF and ADF (Table 6.8). Phytase and xylanase interaction improved final BW and ADG (Table 6.4), and also increased DE intake (Tables 6.8).

Protein Reduction. Reducing dietary protein had no effect on feed intake (ADFI), daily gain (ADG) and Gain:Feed ratio, but improved nitrogen retention (RN), probably due to increased digestibilities for energy and nutrients in the reduced protein diets, or due to more appropriate dietary amino acid pattern. Le Bellego et al. (2001) reported similar performance in pigs fed low protein, amino acid-supplemented diets, and found reduced or unaffected crude nutrient digestibility. In agreement with Le Bellego et al. (2001), we found a tendency for increased energy retention (RE) and dietary net energy (NE) in pigs fed reduced protein diets.

There may be limitations to using LP, amino acid-supplemented diets in a commercial setting. One main constraint is the cost of crystalline amino acids relative to soybean meal (Han and Lee, 2000). Lysine, methionine and threonine generally can be purchased at competitive prices, depending on the current or market price of SBM. The price for other crystalline amino acids is much greater, and it is currently not economical to include them in commercial diets. To obtain large decreases in crude content (i.e., 4 percentage units), diets must often be supplemented with other amino acids, especially tryptophan for C-SBM diets. Smaller decreases, of 2 to 3 percentage units, with

supplementation of several amino acids may also restrict the use of these diets in a commercial setting. Lee et al. (2001) supplemented lysine, methionine and threonine or these three plus tryptophan to LP grower pig diets and found that diet cost per kilogram of weight gain increased by 5.5 and 7.8%, over the intact protein diet. This was in part caused by lower growth rates of pigs fed these two diets compared to pigs fed the control diet. However, one Chinese study reported a lower feed cost to kg gain ratio in 15 kg pigs fed a 15% CP diet, supplemented with amino acids to NRC (1988) levels, compared to an 18% CP control diet (Hsieh and Chiang, 1994).

Aside from cost, there also appears to be a biological limitation to the use of synthetic amino acids. As CP content is reduced, individual amino acids become sequentially deficient. The order in which amino acids become limiting depends greatly on the feedstuffs utilized and the physiological state of the pig. Depending on how severely CP content is decreased, supplementation of one or several synthetic amino acids may be required. Diets decreased in CP content by one to two percentage units generally require only lysine supplementation. LP diets reduced by two percentage units and supplemented with lysine appear to support similar performance as compared to conventional diets for growing pigs (Lee et al., 2001). Decreases beyond 2 percentage units generally require addition of other indispensable amino acids.

The reduction of dietary protein by 3 pu in this study maintained performance (final BW, ADFI, ADG and G:F) with supplementation of lysine and threonine. Reducing dietary protein with no effect of feed intake, daily gain and gain to feed, but improved N retention is possibly due to increased digestibilities of energy and nutrients in the LP, amino acid-supplemented diets. Le Bellego et al. (2001) did not report performance depression in pigs fed LP, amino acid-supplemented diets, but observed reduced or unaffected crude nutrient digestibility. In agreement with Le Bellego et al. (2001), a trend for increased energy retention and dietary NE in pigs fed the LP diet were found in the present study. Contrary to a study in which a 3 pu decrease in CP resulted in inferior performance in growing pigs when limiting amino acids and nonessential nitrogen appeared to be sufficient (Tuitoek et al., 1997a). Rather, decrease in average daily gain

and increase backfat thickness in growing pigs (Schoenherr, 1992; Tuitoek et al., 1997a) have resulted CP reductions of 4 pu and at this level, supplementation of several amino acids including lysine, threonine, tryptophan, methionine and possibly isoleucine and valine are required.

From our N balance studies, the dietary protein reduction lowered N intake, and concomitantly resulting in reduced fecal and urine N excretion, without affecting retained N (g/d). Unlike studies where severe reductions in CP (i.e., 4% or greater) may limit N retention, possibly due to a deficiency of nonspecific nitrogen (Kerr and Easter, 1995) and an imbalance of indispensable to dispensable amino acids, N retention did not vary between the HP and LP diets. The increase in retained N resulting from CP reduction in our study was marginal and might have contributed the higher efficiency of utilization of N for protein deposition (kn or percent retained N). Decreases greater than 4 percentage units may be possible; however, provided that the proper amino acids are supplemented (Lenis and Jongbloed, 1999). To achieve this, further understanding of ideal protein ratios and whole body requirements for amino acids and nitrogen in sows and growing pigs is essential.

The effect of dietary protein reduction on fecal and urinary carbon followed a similar pattern to that observed with fecal and urinary N, whilst intake of carbon was similar, possibly due to better utilization of the carbon skeletons in the LP diet from the supplemented amino acids, as opposed to that from protein-bound amino acids. There are no comparable studies in the literature reporting on associated effects of CP reduction on carbon excretion, except for other studies reported in this thesis (Chapters 4 and 5). These results are in consonance with the increased DE and ME intakes (MJ/kg), increased digestibility of GE, fiber (NDF and ADF) and carbon. Considering that fermentable fiber represents the difference between digestible organic matter (DOM) and the sum of digestible CP, digestible crude fat, starch and sugars, a mathematical relationship confirming the connection between enteric fermentation and CH_4 production was established (Rijnen, 2003; Rijnen et al., 2003). We also had previously demonstrated a reduction in dietary protein content reduced CH_4 emission in growing pigs (Atakora et

al., 2003). Whilst in that study diet protein level was confounded with diet level suggesting a possibility of both fermentable carbon as well as the fermentable carbon to nitrogen ratio influences the extent of microbial fermentation and CH_4 emission in pigs, in the current study in which diet protein and fiber levels were not confounded energy losses in CH_4 emission did not reach significance ($P = 0.11$; Table 6.9) and CH_4E:DE were not affected ($P = 0.10$; Table 6.11). In comparison to poultry, given the short retention time of digesta in the digestive tract and the less-developed hindgut, microbial fermentation and CH_4 emission will have a smaller impact on energy metabolism in poultry.than in swine (Moran, 1982 cited in de Lange and Birkett, 2005; Moughan et al., 2000 cited in de Lange and Birkett, 2005).

Phosphorus Reduction. Within the LP, amino acid supplemented diet, the LP+ (negative control), was higher in phosphorus than the LP-. The reduction in phosphorus was the result of omission of dicalcium phosphate from the LP+ diet. Few parameters responded significantly to P reduction, however, most showed consistent directions of response. In agreement with Brady et al. (2002), P reduction significantly impaired ADG and reduced nutrient digestibility numerically. P reduction increased heat production (HE) and numerically reduced dietary DE, ME and NE. The lack of significant effects was probably a result of the moderate P reduction (13% below diet Con).

Phytase Supplementation. Phytic acid is the major P storage compound of most seeds and cereal grains. In wheat, phytic acid is mostly contained in the bran, which contains 5% phytic acid (Garcia-Estepa et al., 1999). Wheat contains 0.32% phytate, with approximately 87% of it contained in the aleurone layer, 13% in the germ, 2% in the endosperm, and 0% in the hull (O'Dell et al., 1972). Phytic acid can form complexes with multivalent cations such as Ca, Mg, Zn, and Fe, starch, free AA, and proteins (Selle et al., 2000) and thus exists in many forms. Most of the P in plant-based feedstuffs is present as phytate P (Liao et al., 2005). Another form is phytin, which is the Ca and Mg salt of phytic acid (Oatway et al., 2001). Phytate chelates mineral (including calcium and phosphorus), and their complexes formed are generally insoluble at physiological pH (Ravindran et al., 1994), and thus bound minerals are not available to swine.

Phytase inclusion improved P digestibility as expected, which probably reversed the negative effect of phosphorus reduction on ADG. The non-significant increase in dietary DE, ME and NE with phytase supplementation is in accord with Kies et al. (2005). Phytase improved feed GE content and the digestibility of carbon, NDF and ADF. Adding phytase to feeds containing phytate can catalyze the removal of the orthophosphate group from phytate (Maga, 1982), thereby releasing the bound nutrients and improving nutrient digestibility. Phytic acid binds the main energy macronutrient for swine, starch, through H bonding (Oatway et al., 2001). Pigs have a limited ability to digest phytate P, because endogenous phytate necessary for hydrolysis of phytate is lacking (Golovan et al., 2001).

Effects of phytase on energy digestibility of swine diets are rarely studied. Supplementing phytase to rice bran-based diets either low or high in phytate has been shown to not affect apparent ileal digestibility of GE in grower pigs (Liao et al., 2005). Dietary calcium and available phosphorus content of the experimental diets might affect phytase efficacy. The combination of a reduced calcium and phosphorus content and phytase supplementation increased nutrient and energy digestibility in diets for pigs (Johnston et al., 2004). The improved feed GE content with phytase in this study indicates that complexes between phytic acid and macronutrients in wheat-based diets (with middlings) are significant and that energy is less available without phytase supplementation.

The effects of phytase on phosphorus digestibility or availability in plant-based feedstuffs have been well documented. In young pigs, phytase has been shown to increase phosphorus availability (Yi et al., 1996b), and an *Escherichia coli*-derived phytase has been shown to improve calcium and phosphorus digestibility and retention (Adeola et al., 2004). In this study, phytase supplementation improved phosphorus digestibility, which possibly reversed or prevented any negative effect of phosphorus reduction on ADG. The improved phosphorus digestibility reflected in lowered amount of excreted P in feces.

Furthermore, in this experiment, phytase supplementation to the LP- diet lowered intake and excretion of Ca in relationship to the improved digestibility of phosphorus, indicating that phosphorus is being liberated from phytate-P and phytin.

Phytase and Xylanase Interaction. Xylanase and phytase interacted positively on final BW, but the effect of the interaction on ADG was a trend ($P = 0.06$), suggesting that phytase and xylanase did not act independently to influence these growth parameters (Table 6.4). Compared to the diet LP-, phytase and xylanase individually improved final weight by 1.7 kg and 1.9 kg respectively, whilst the phytase-xylanase interaction improved the final weight by 1.2 kg (Table 6.4). These improvements might have also resulted from the improvement in ME intake (MJ/kg) in this study (Table 6.8). This possibly occurred because supplemental xylanase disrupts the cell wall matrix and hydrolyzes otherwise unavailable carbohydrates, while at the same time allowing the supplemental phytase to gain access to phytate-bound nutrients like P, proteins, and starch (Oryschak et al., 2002). Such improvements cause by phytase-xylanase interaction might have also been caused by changes in passage rate of digesta, thereby allowing a prolonged contact time of the phytase with its substrate at the optimum pH.

The combined addition of phytase and xylanase did not improve any of response variable beyond that obtained with either enzyme alone. This lack of an additive effect is contrary to the results of Oryschak et al. (2002), but may be due to the low content (8.8%) of NSP in the wheat used in this trial. Kim et al. (2005) indicated that wheat quality may affect the response of nutrient digestibility to phytase and/or xylanase addition.

Xylanase Supplementation. The NSP-degrading enzymes have had inconsistent effects on growth performance in swine (Bedford and Schulze, 1998). For example, Zijlstra et al. (2004) found that supplementation of a NSP-degrading enzyme to a wheat and canola meal-based diet, improved ADG as a result of improved ADFI. Improved ADG has also been attributed to improved G:F (Bedford et al., 1992; van Lunen and Schulze, 1996). In contrast, xylanase supplementation to an LP, amino acid-supplemented wheat-wheat middlings based diet did not affect any of the performance parameters (final BW, ADFI,

ADG and G:F) in this study. Xylanase supplementation maintained final BW similar to the pigs fed the Con (positive control) diet.

Xylanase did not affect any of the parameters of N balance and most of the parameters of carbon balance; however xylanase supplementation, lowered ($P = 0.05$) carbon retention by 2.3 units in this study, with an improvement in the digestibility of carbon of 1 pu. This increase in fecal carbon digestibility (Table 6.8), but lower carbon retention (Table 6.6) was due to increased carbon excreted as CH_4 (Table 6.9). In this study, xylanase supplementation improved ($P = 0.02$) measured DE intake, but not measured ME intake (MJ/kg), improved digestibility of GE, carbon, crude fat, NDF and ADF (Table 6.8). These differences are due to the increase in hindgut fermentation as shown by the increase in CH_4 excretion.

Xylanase addition improved nutrient digestibility, DE and ME, but not dietary NE in this experiment. We observed a tendency ($P = 0.09$) for increased methane emission from xylanase supplemented diets, which will attenuate the effects of xylanase when moving from DE to ME and NE. By-products of cereal grains have a high content of NSP (Slominski et al., 2004). Pigs do not produce endogenous enzymes to digest NSP; therefore supplementation of NSP-degrading enzymes in high-NSP diets is one approach to reduce detrimental effects of NSP and improve the nutritional value for young pigs (Li et al., 1996). In diets containing wheat bran, NSP-degrading enzymes have been found to increase soluble saccharides in the stomach and small intestine, and increase VFA in the ileum (van der Meulen et al., 2001), indicating that NSP-degrading enzymes shift NSP digestion partially from the hindgut to the small intestine. The NSP-degrading enzymes thus can improve energy utilization of high-NSP diets in young pigs (Graham et al., 1986).

We observed a trend ($P = 0.09$; Table 6.11) for increased emission of methane (g/d) and the CO_2-equivalent due to methane alone from xylanase-supplemented diet, which will attenuate the effects of xylanase when moving from DE to ME and NE, supporting a shift of fermentable carbohydrate digestion from the hindgut into the smaller intestine. This

significant decrease in CH_4 emission translated to a corresponding significant reduction in total GHG expressed as CO_2-equivalent from combined CO_2 and and CH_4. The combined addition of phytase and xylanase did not improve any response parameter beyond that obtained with either enzyme alone. This lack of an additive effect is contrary to the results of Oryschak et al. (2002), but may be due to the low content (8.8%) of non-starch polysaccharides in the wheat used in this trial. Kim et al. (2005) indicated that wheat quality may affect the response of nutrient digestibility to phytase and/or xylanase addition.

Xylanase improved apparent AA digestibility, similar to improved AA digestibility in wheat-based diets fed to grower pigs (Barrera et al., 2004), indicating that wheat NSP hamper AA digestibility. The arabinoxylans enclose AA in the grain, thus directly interfering with AA digestion and absorption in the small intestine, or enhance secretion of endogenous AA. In this study, xylanase supplementation improved nitrogen digestibility and might have contributed to the improved percent nitrogen retention resulting from a protein effect (Table 6.4).

Xylanase supplementation to wheat-wheat middlings diet did not affect P digestibility. In mature cereal grains, a large portion of the P is present as phytate-bound P (Ravindran et al., 1994). Bran and kernel layers of wheat are major storage sites of phytate and P (Maga, 1982), the same sites contain arabinoxylans, which are the major substrates for xylanase. The absence of xylanase effect on P digestibility did not result in an expected indirect benefit of xylanase, because P, either bound or not bound to phytate would have been better exposed to digestive enzymes or supplemental phytase.

6.5 Conclusion

Dietary protein reduction maintained animal performance in this study. Reducing dietary protein lowered nitrogen excretion and increased N retention. The reduction of dietary phosphorus did not impair energy metabolism as shown by an increased heat production. Negative effects of reduced phosphorus were reversed by phytase supplementation to diets. In this experiment, phytase and xylanase addition to diets were not additive.

However, phytase and xylanase interacted to improve final body weight and showed potential to improve daily gain.

Both phytase and xylanase improved the digestibility of nutrients, end weight, and ADG of growing-finishing pigs fed a diet that was formulated to be marginal in terms of crude protein and phosphorus.

6.6 Implications

Potentially, the best diets to minimize the environmental concerns regarding the excretion of large quantities of phosphorus in effluent from intensive pig production operations will require the use of well formulated low protein amino acid supplemented diets without supplemental phosphate, and routinely supplemented with phytase or xylanase. A combination of the above measures should result in best utilization of dietary energy and phosphorus. By products (wheat middlings) could become more valuable feed ingredients if the nutrients bound by arabinoxylans and phytate were made available for use by the pig through enzyme supplementation. Wheat middlings contain digestible nutrients and should thus be considered a better feedstuff for swine, with the use of appropriate enzymes.

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Table 6.1. Ingredient (g/kg) and nutrient (g/kg) composition of diets

Ingredient	Six experimental diets[1]					
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl[2]**	**Xyl**
Wheat	755.2	866.5	868.3	868.6	868.3	868.3
Wheat middlings	100.0	100.0	100.0	100.0	100.0	100.0
Canola oil	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal	117.0	0.0	0.0	0.0	0.0	0.0
L-lysine HCl	0.0	3.3	3.3	3.3	3.3	3.3
L-threonine	0.0	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	2.4	4.3	0.0	0.0	0.0	0.0
Limestone	10.4	10.1	12.6	12.6	12.6	12.6
$NaHCO_3$	2.0	3.3	3.3	3.3	3.3	3.3
NaCl	1.0	0.0	0.0	0.0	0.0	0.0
Vit.-Min. premix[3]	2.0	2.0	2.0	2.0	2.0	2.0
Phytase[4], units	0.0	0.0	0.0	500.0	500.0	0.0
Xylanase[5], units	0.0	0.0	0.0	0.0	4000.0	4000.0
Calculated nutrients						
ME, MJ/kg	13.1	13.2	13.2	13.2	13.2	13.2
CP	177.0	141.0	142.0	142.0	142.0	142.0
Lysine	6.1	6.1	6.1	6.1	6.1	6.1
Methionine	2.6	2.2	2.2	2.2	2.2	2.2
Threonine	5.2	4.3	4.3	4.3	4.3	4.3
Calcium	5.4	5.4	5.4	5.4	5.4	5.4
P (total)	4.9	4.8	4.1	4.1	4.1	4.1
P (avail)	1.9	2.1	1.3	1.3	1.3	1.3
NDF	153.0	153.0	153.0	153.0	153.0	153.0
ADF	51.9	45.4	45.4	45.4	45.4	45.4
mEq[6]	191.9	124.7	124.9	124.9	124.9	124.9
Cost[7], Can$/kg	0.31	0.30	0.30	0.30	0.30	0.30

[1]Experimental diets: Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = Low protein-low phosphorus with phytase alone; PhyXyl = Low protein-low phosphorus with combined phytase and xylanase; Xyl = Low protein-low phosphorus with xylanase alone.

[2]Phyzyme XP5000 plus Porcyme 9300 - combined phytase and xylanase source, included at 100 g/tonne and 1000 g/tonne, supplied 500 and 4000 units of individual enzymes respectively per kg diet.

[3]Vitamin-Mineral premix: Same as used in previous experiment (Table 5.2).

[4]Phyzyme XP5000 - phytase source, included at 100 g/tonne, supplied 500 phytase units per kg diet.

[5]Porzyme 9300 - xylanase source, included at 1000 g/tonne, supplied 4000 xylanase units per kg diet.

[6]Milliequivalents of cation-anion difference.

[7]Cost of enzymes not included.

Table 6.2. Analysed nutrient composition (g/kg) of experimental diets

Nutrient	Six experimental diets[1]					
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl**	**Xyl**
Dry matter	866.0	865.0	868.0	872.0	869.0	872.0
Crude protein[2]	190.0	162.0	162.0	159.0	156.0	159.0
Lysine	7.4	6.6	7.0	6.8	6.6	6.7
Crude fat	26.0	28.0	27.0	27.0	27.0	27.0
Crude ash	37.0	36.0	31.0	30.0	31.0	33.0
Neutral detergent fibre	179.0	190.0	169.0	191.0	199.0	168.0
Acid detergent fibre	45.0	50.0	42.0	48.0	49.0	43.0
Carbon	432.0	428.0	429.0	430.0	430.0	428.0
Phosphorus	5.1	5.4	4.7	4.6	4.5	4.4
Calcium	7.7	7.8	6.9	6.0	6.4	7.5

[1]Experimental diets: Con = High protein, high phosphorus diet; LP+ = Low protein, high phosphorus diet; LP- = Low protein, low phosphorus diet; Phy = LP- plus phytase alone; PhyXyl = LP- plus combined phytase and xylanase; Xyl = LP- plus xylanase alone.

[2]Calculated as % N x 6.25.

Table 6.3. Mean chemical composition (%) of feces and urine from growing-finishing pigs fed wheat-based diets ad libitum

Parameter	Six experimental diets[1]					
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl**	**Xyl**
Feces						
Nitrogen	3.1	2.6	2.7	2.6	2.5	2.5
Crude fat	2.9	2.5	2.7	2.6	2.5	2.5
Crude ash	17.2	16.5	16.0	13.9	15.0	15.5
Neutral detergent fibre	56.2	57.4	57.6	58.0	57.2	58.2
Acid detergent fibre	24.3	24.3	24.7	24.9	25.0	24.8
Carbon	43.5	43.9	44.5	45.1	44.9	44.2
Phosphorus	2.3	2.2	2.0	1.7	1.7	2.0
Calcium	2.4	2.3	2.1	1.8	1.9	2.3
Gross energy, kcal/kg	4375.8	4383.2	4443.5	4530.9	4460.1	4400.2
Urine						
Nitrogen	1.5	2.1	1.7	1.8	2.2	1.6
Crude protein[2]	9.6	13.0	10.8	11.4	13.2	10.2

[1]Experimental diets: Con = High protein, high phosphorus diet; LP+ = Low protein, high phosphorus diet; LP- = Low protein, low phosphorus diet; Phy = LP- plus phytase alone; PhyXyl = LP- plus combined phytase and xylanase; Xyl = LP- plus xylanase alone.

[2]Crude protein = % N x 6.25

Table 6.4. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on growth performance in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl**	**Xyl**		**Prot**	**Phos**	**Phy**	**Phy *Xyl**	**Xyl**
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
End wt., kg	74.2	73.2	72.0[a]	73.7[ab]	73.2[b]	73.9[ab]	1.2	0.18	0.12	0.44	**0.05**	0.29
ADFI,[3] g/d	2344	2291	2180	2249	2267	2280	103	0.20	0.39	0.69	0.57	0.46
ADG,[4] g/d	746	737	666	745	727	763	42	0.13	0.14	0.47	***0.06***	0.23
Gain:Feed	0.32	0.32	0.32	0.32	0.30	0.34	0.02	0.74	0.55	0.24	0.21	0.89

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Feed intake.

[4]Daily gain.

[a,b]Different letters in a row denote differences at $P < 0.05$.

Table 6.5. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on nitrogen balance in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl**	**Xyl**		**Prot**	**Phos**	**Phy**	**Phy *Xyl**	**Xyl**
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
Intake N,[3] g/d	72.2	59.0	57.3	57.8	58.5	56.4	2.7	**<0.0001**	0.91	0.53	0.69	0.97
Feces N,[4] g/d	5.80	4.00	4.28	4.12	3.92	3.68	0.4	**0.0002**	0.42	0.89	0.44	0.17
Urine N,[5] g/d	44.1	30.5	29.6	30.0	31.1	30.2	1.3	**<0.0001**	0.62	0.57	0.81	0.43
Retained N,[6] g/d	22.3	24.3	23.5	23.3	23.6	22.8	1.7	0.49	0.90	0.77	0.61	0.86
Retained N, %	30.2	41.0	41.0	40.2	40.1	40.2	2.0	**0.001**	0.89	0.81	0.88	0.86

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl); and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Nitrogen intake.

[4]Feces nitrogen.

[5]Urine nitrogen

[6]Retained nitrogen.

Table 6.6. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on parameters of carbon balance (g/d) in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	LP+	LP-	Phy	PhyXyl	Xyl		Prot	Phos	Phy	Phy *Xyl	Xyl
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
Intake C,[3]	1029	958	948	976	990	967	45	0.13	0.91	0.45	0.93	0.65
Feces C,[4]	81.5	68.5	71.5	72.6	70.8	64.9	5.3	**0.04**	0.55	0.38	0.55	0.34
Urine C,[5]	39.7	27.4	26.6	27.0	28.0	27.2	1.2	**<0.0001**	0.62	0.57	0.81	0.43
CO_2 emission	2212	2048	2034	1990	2130	2180	88	0.14	0.83	0.55	0.97	0.11
CH_4 emission	23.8	20.5	21.4^{ab}	18.9^{a}	22.0^{ab}	23.5^{b}	1.5	0.13	0.83	0.19	0.75	***0.09***
Retained C,[6]	288	298	279	323	283	262	37	0.81	0.78	0.27	0.70	0.38
Retained C, %	28.0	31.1	29.4	33.1	28.6	27.1	3.1	0.79	0.81	0.38	0.90	0.40

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Urine carbon.

[4]Feces carbon.

[5]Urine carbon.

[6]Retained carbon = Digestible carbon intake – (gCO_2d*12/44) – (gCH_4d*12/16) – urinary carbon – fecal carbon.

[a,b]Different letters in a row denote differences at $P < 0.05$.

Table 6.7. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on energy metabolism in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	LP+	LP-	Phy	PhyXyl	Xyl		Prot	Phos	Phy	Phy *Xyl	Xyl
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
ME intake, MJ/d	31.8	32.1	30.8	32.4	31.8	31.9	1.6	0.64	0.67	0.51	0.48	0.84
HE,[3] MJ/d	19.0	18.3	19.9	19.4	20.3	20.9	1.1	0.39	0.87	0.57	0.95	0.36
RE,[4] MJ/d	12.8	13.7	12.9	14.9	13.0	12.1	1.7	0.81	0.78	0.27	0.70	0.38
RE:IME[5]	0.40	0.43	0.41	0.45	0.39	0.37	0.04	0.62	0.90	0.38	0.22	0.75
NE, MJ/kg	9.9	10.7	10.5	11.1	10.3	10.0	0.5	0.26	0.97	0.37	0.68	0.17
RQ[6]	1.28	1.13	1.18	1.16	1.18	1.21	0.08	0.19	0.64	0.71	0.88	0.74

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Heat production calculated from Brouwer (1965) formula.

[4]Retained energy = Retained protein energy (RPE) + Retained fat energy (RFE).

[5]Retained energy to ME intake ratio.

[6]Respiration quotient = Volume of CO_2 emission/Volume of O_2 consumption.

Table 6.8. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on measured DE and ME (MJ/kg) and digestibility (%) of GE and nutrients in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	**LP+**	**LP-**	**Phy**	**PhyXyl**	**Xyl**		**Prot**	**Phos**	**Phy**	**Phy *Xyl**	**Xyl**
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
DE,[3] MJ/kg	15.0	15.2	15.1^{a}	15.2^{ab}	15.3^{b}	15.4^{b}	0.08	**0.05**	0.51	0.87	**0.05**	**0.02**
ME,[4] MJ/kg	13.5	14.0	14.0^{a}	14.2^{b}	14.1^{ab}	14.1^{ab}	0.09	**0.0002**	0.62	0.40	**0.03**	0.60
Digestibility, %												
GE[5]	91.2	92.3	91.8^{a}	91.9^{a}	92.2^{ab}	92.8^{b}	0.5	**0.04**	0.27	0.38	0.26	**0.04**
Nitrogen	92.0	93.1	92.5^{a}	92.9^{ab}	93.2^{ab}	93.6^{b}	0.5	**0.03**	0.20	0.98	0.28	**0.05**
Carbon	92.0	92.9	92.4^{a}	92.5^{a}	92.8^{ab}	93.4^{b}	0.4	***0.07***	0.27	0.37	0.22	**0.05**
Calcium	75.0	79.6	77.7	79.4	79.2	80.0	1.5	**0.02**	0.27	0.63	0.18	0.32
Phosphorus	63.2	71.5	68.4^{a}	73.8^{b}	74.1^{b}	70.1^{a}	1.7	**0.0002**	0.11	**0.001**	0.52	0.41
Crude fat	90.4	94.0	92.5^{a}	93.2^{ab}	93.8^{b}	94.0^{b}	0.5	**0.001**	*0.08*	0.52	0.31	**0.03**
NDF[6]	75.1	79.1	75.3^{a}	78.5^{b}	80.0^{ab}	77.6^{b}	1.3	**0.005**	**0.005**	**0.001**	0.61	**0.04**
ADF[7]	56.5	66.5	56.9^{a}	63.4^{b}	64.4^{b}	62.9^{b}	2.0	**0.0001**	**0.0002**	**0.008**	***0.08***	**0.03**

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Measured digestible energy.

[4]Measured metabolizable energy.

[5]Gross energy.

[6]Neutral detergent fiber.

[7]Acid detergent fiber.

[a,b,c,d]Different letters in a row denote differences at $P < 0.05$.

Table 6.9. Effects of dietary protein and phosphorus level, phytase, xylanase, and combined phytase-xylanase on partitioning of dietary energy (MJ/kg) in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	LP+	LP-	Phy	PhyXyl	Xyl		Prot	Phos	Phy	Phy *Xyl	Xyl
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
FE,[3] MJ/kg	3.40	2.90	2.99	3.06	2.94	2.70	0.2	**0.04**	0.54	0.38	0.61	0.28
DE,[4] MJ/kg	15.0	15.2	15.1^{a}	15.2^{ab}	15.3^{b}	15.4^{b}	0.08	**0.05**	0.51	0.87	**0.05**	**0.02**
ME,[5] MJ/kg	13.5	14.0	14.0^{a}	14.2^{bc}	14.1^{ab}	14.1^{bc}	0.09	**0.0002**	0.62	0.40	**0.03**	0.60
CH_4E,[6] MJ/kg	1.30	1.10	1.19^{a}	1.05^{a}	1.22^{ab}	1.30^{ab}	0.08	0.13	0.83	0.19	0.75	***0.09***
UE,[7] MJ/kg	2.00	1.40	1.37	1.39	1.44	1.40	0.06	**<0.0001**	0.62	0.57	0.81	0.43
NE,[8] MJ/kg	9.9	10.7	10.5	11.1	10.3	10.0	0.5	0.26	0.97	0.37	0.68	0.17
HI,[9] MJ/kg	9.6	9.4	9.4	8.9	9.7	10.3	1.1	0.88	0.99	0.57	0.98	0.40

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Feces energy.

[4]Measured digestible energy.

[5]Measured metabolizable energy.

[6]Methane energy = 39.56*LCH4d*0.001.

[7]Urinary energy = (0.333*Urinary carbon + 0.093*Urinary nitrogen)/100.

[8]Net energy = (MEm + RPE + RFE)/(0.001*Feed intake).

[9]Heat increment = Heat production – MEm.

[a,b,c]Different letters in a row denote differences at $P < 0.05$.

Table 6.10. Effects of dietary protein and phosphorus level on relative energy losses of energy intake (%) and efficiency of nutrient and energy utilization in growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SEM	*P*-values[2]				
	Con	LP+	LP-	Phy	PhyXyl	Xyl		Prot	Phos	Phy	Phy *Xyl	Xyl
Energy losses[3]												
Feces	18.7	15.6	16.1	16.1	15.8	14.7	0.01	**0.03**	0.64	0.55	0.55	0.40
Urine	11.2	7.7	7.4	7.3	7.7	7.6	0.003	**<0.0001**	0.46	0.87	0.76	0.25
Methane	7.2	6.2	6.4^{ab}	5.5^{a}	6.5^{ab}	7.1^{b}	0.004	0.11	0.93	0.13	0.72	***0.06***
Nutrient[4]												
kn	0.31	0.41	0.41	0.40	0.40	0.40	0.02	**0.001**	0.89	0.81	0.88	0.86
kpf	0.40	0.43	0.41	0.45	0.39	0.37	0.03	0.62	0.90	0.38	0.75	0.22
Energy[5]												
DE:GE	0.82	0.83	0.81^{b}	0.80^{a}	0.82^{b}	0.83^{b}	0.006	0.36	**0.04**	**0.01**	0.56	**0.001**
ME:GE	0.83	0.87	0.85	0.85	0.85	0.86	0.005	**0.0002**	**0.04**	0.96	0.11	0.70
ME:DE	0.91	0.93	0.92	0.93	0.92	0.92	0.003	**<0.0001**	0.85	0.31	0.57	0.26
UE:DE	0.14	0.09	0.09	0.09	0.09	0.09	0.004	**<0.0001**	0.67	0.61	0.68	0.58
CH_4E:DE	0.09	0.08	0.08^{ab}	0.07^{a}	0.08^{ab}	0.09^{b}	0.004	***0.10***	0.83	0.19	0.65	***0.10***
NE:ME	0.73	0.76	0.75	0.78	0.73	0.71	0.03	0.54	0.88	0.44	0.88	0.13
HE:ME	0.63	0.64	0.66	0.63	0.64	0.64	0.03	0.88	0.88	0.66	0.66	0.86
RE:ME	0.40	0.43	0.41	0.45	0.39	0.37	0.03	0.62	0.90	0.38	0.75	0.22

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase; Xyl = LP- plus xylanase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Phytase (Phy), Xylanase (Xyl), and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]Energy losses expressed as percentage of GE intake.

[4]Efficiency of: nitrogen utilization for protein deposition (kn) and tissue deposition (kpf).

[5]GE = Gross energy, FE = Feces energy, DE = Digestible energy, UE = Urine energy, CH_4E = Methane energy, HE = Heat production, NE = Net energy, RE = Retained energy.

[a,b]Different letters in a row denote differences at $P < 0.05$.

Table 6.11. Effects of dietary protein and phosphorus level, xylanase, phytase, and combined xylanase-phytase on O_2 consumption (g/d), CO_2 and CH_4 emission (g/d), CO_2-equivalent (g/d), respiration quotient, heat production and heat increment (MJ/d) of growing-finishing pigs fed wheat-based diets *ad libitum*

Parameter	Experimental diets[1]						SE	*P*-values[2]				
	Con	LP+	LP-	Phy	Phy +Xyl	Xyl		Prot	Phos	Phy	Phy *Xyl	Xyl
No. of pigs	12	12	12	12	12	12	---	---	---	---	---	---
O_2 consumption	1241	1295	1275	1240	1272	1392	163	0.81	0.94	0.58	0.77	0.64
CO_2 emission	2212	2048	2034	1990	2130	2180	88	0.14	0.83	0.55	0.97	0.11
CH_4 emission	23.8	20.5	21.4^{ab}	18.9^{a}	22.0^{ab}	23.5^{b}	1.5	0.13	0.83	0.19	0.75	***0.09***
CO_2-eq[3] (CO_2)	2212	2048	2034	1990	2130	2180	88	0.14	0.83	0.55	0.97	0.11
CO_2-eq[4] (CH_4)	1377	1183	1236^{ab}	1093^{a}	1272^{ab}	1358^{b}	86	0.13	0.83	0.19	0.75	***0.09***
CO_2-eq[5]	3589	3229	3282^{ab}	3089^{a}	3403^{ab}	3530^{b}	149	***0.08***	0.19	0.28	0.82	***0.08***
RQ[6]	1.30	1.10	1.18	1.16	1.18	1.21	0.08	0.19	0.64	0.71	0.88	0.74
HE[7]	19.0	18.3	19.9	19.4	20.3	20.8	1.1	0.39	0.87	0.57	0.95	0.36
HI[8]	9.6	9.4	9.4	8.9	9.7	10.3	1.1	0.88	0.99	0.57	0.98	0.40

[1]Values are least square means of experimental diets without or with enzyme supplementation from Proc Mixed procedures (SAS, 2005). Con = High protein-high phosphorus (Positive control) diet; LP+ = Low protein-high phosphorus diet; LP- = Low protein-low phosphorus (Negative control) diet; Xyl = LP- plus xylanase; Phy = LP- plus phytase; Xyl+Phy = LP- plus combined xylanase-phytase.

[2]Probability values for Protein effect (Prot) = Con vs. LP+; Phosphorus effect (Phos) = LP+ vs. LP-; Xylanase (Xyl), Phytase (Phy) and Xyl*Phy interaction effects from a 2x2 (+2) factorial arrangement in randomized complete block design analysis (SAS, 2005).

[3]CO_2-equivalent based CO_2 emission from pig, using GWP = 1 for CO_2 (IPCC, 1997).

[4]CO_2-equivalent based CH_4 emission from pig, using GWP = 21 for CH_4 (IPCC, 1997).

[5]CO_2-equivalent based CO_2 plus CH_4 emissions from pig, using GWP 1 and 21 for CO_2 and CH_4, respectively (IPCC, 1997).

[6]Respiration quotient = CO_2 emission (L/d)/ O_2 consumption (L/d).

[7]Heat production = Calculated using Brouwer (1965) formula.

[8]Heat increment = HE minus ME (for maintenance).

7.0 SUMMARY, GENERAL DISCUSSION AND FUTURE DIRECTIONS

For the effective use of LP, amino acid-supplemented diets involving the use of different feed ingredients for the various classes of swine (and at different physiological stages/states), it is important that the feeding value of such diets is properly investigated. Insufficient knowledge and inaccurate evaluation of the feeding value of diets of different composition will result in variable and often poorer than anticipated animal responses when manipulating diet ingredients composition, even when target available nutrient levels are maintained. Alternatively, this may increase the nutrient costs of animal feeds, as a result of raising safety margins in target nutrient levels, or increase the ingredient cost of animal feed, as a result of the inclusion of very expensive ingredients, especially some of the synthetic amino acids. As feed energy is generally the largest single cost factor in animal production, special consideration should be given to the useful energy or bio-available content of ingredients and ingredient-specific effects on utilization of useful energy of diet ingredients.

From the sow experiments (Experiment 1) the CO_2 production of sows fed LP diet was 6% greater for non-pregnant sows during maintenance, whilst it was between 2.5 and 5.4% lower for sows during gestation and lactation (Table 3-9). Typically, sows spend about one week out of 5 months neither pregnant nor lactating, thus it can be concluded that a reduction of dietary protein contents will reduce CO_2 production. The CH_4 production from non-pregnant sows was found to be 0.821 g/MJ metabolizable energy intake for the HP, and 0.558 g/MJ metabolizable energy intake for the LP diet. Yearly estimated CO_2-equivalents (Table 3-9) were based on the following assumptions: gestation period of 115 d, 7 d return to oestrus, 9 d for 20% of the sow herd not inseminated successfully at the first oestrus after weaning, and duration of lactation as 23 d. These assumptions calculate to154 d per parity or 2.37 parities per year. Therefore, the CO_2-equivalents by sow and year are 2015 kg for HP, and 1682.4 kg for LP fed sow, a reduction of 16.6% (Table 3-9). Thus, a 20% reduction in dietary protein content was estimated to reduce CO_2-equivalents emitted per sow per year by 333.5 kg (from a population of 1000 sows). The results from this study indicate the impact of CH4 in energy research cannot be ignored as in previous studies. The benefits of improved

energy efficiency and reduced N excretion associated with feeding LP diets should be an incentive to facilitate the commercial adoption of feeding of LP diets to sows.

The reduction of protein concentration in diets based on either WBC or CS for non-pregnant sows, fed during maintenance tended to increase CO_2 emissions. Reduction of dietary protein was associated with lower NDF contents, and thus reduced CH_4 production in non-pregnant sows. The WBC-LP diet led to a significant reduction in CH_4 production, whilst the CH_4 production was not affected by protein level in the corn-soybean meal diets. The CO_2-equivalents emitted by non-pregnant sows was lower for WBC-LP diet than WBC-HP diet. The protein reduction in WBC diets had no effect in the corn-soybean meal diets. Overall, the CO_2-equivalents were reduced by 16.4% with the reduction of dietary protein concentration.

Reduction of dietary protein concentration for gestating sows had no statistically significant impact on animal performance. However, piglet weight at birth and weaning was non-significantly lower when LP diets were fed to lactating sows. The number of piglets weaned tended to be lower for the LP treatment. The CO_2 production by sows during gestation and lactation (with litter) was lower by 5.4% ($P = 0.01$) and 2.5% ($P = 0.1$), respectively. In the absence of CH_4 measurements, the effect of protein reduction on CH_4 production was assumed to be similar to that from non-pregnant sows fed barley-canola diets during maintenance. With this assumption, the reduction of dietary protein concentration reduced CO_2-equivalents emitted from sows by 8%. This estimate, however, does not include emissions from manure, which are related to the nutrient contents of manure. Reducing dietary protein concentration had no effect on fecal carbon excretion. The urinary nitrogen excretion, and concomitantly the carbon excretion, was reduced by 26% when sows were fed LP diets. The total nitrogen excretion from sow within a year was reduced by 20%, when LP diets were fed, while carbon excretion was reduced by 6%.

From Experiment 2, reducing the dietary protein reduced N excretion and the CH_4 emission by pigs, but led to only a marginal reduction in CO_2 production. The CO_2-

equivalents arising from the pigs (animals) themselves – from CO_2 and CH_4 production – tended to be lower with reduced dietary protein. Further GHG emission will depend on the conversion of the carbon and N excreted into manure into CO_2, CH_4, and N_2O. Our results show that reducing dietary protein intake reduces the N excretion by pigs and has the potential to reduce GHG emissions by pigs by over 20%. To realize the full potential, the conversion of nutrients in manure into GHG must be investigated further, along with means to influence this conversion.

The RQ value of 1.14 (4 h) and 1.11 (based on 24 h extrapolated values) indicate that during the feeding period, dietary carbohydrates were the main energy source, sufficient to cover energy requirement without oxidation of fat (Table 4-5). Such RQ values for 4 h compared favorably with values obtained during 24 h respiration studies (Chwalibog et al., 2004). Under normal feeding conditions, the main source of energy for growing-finishing pigs is dietary carbohydrates followed by protein, and provided there is enough energy from carbohydrate and protein to sustain requirements for maintenance and growth dietary fat is not oxidized but retained in the body (Chwalibog and Thorbek, 2000). However, during post-absorption mobilized body fat becomes the main source of energy followed by body protein oxidation. This situation is changed during re-feeding as macronutrients are again available from the diet. Consequently, the metabolism will change from utilizing body fat and protein reserves to using dietary nutrients. The switch between endogenous and exogenous substrates is not an immediate process and a certain time is necessary to re-establish metabolism from catabolic to anabolic conditions. Our results are based on measurement of oxygen consumption and CO_2 emission by indirect calorimetry and urinary nitrogen excretion are in agreement with calculations based gas exchange measurements (Jebb et al., 1996), but can be biased by the potential pitfalls in the interpretation of data from gas exchange measurements (Elia and Livesey, 1988; Ferrannini, 1988; Chwalibog and Thorbek, 2000) if standard error or measurements are not less than ±1.5% compared to the standards. In addition, errors in collection of urine and unaccounted for evaporation of ammonia may cause an underestimation of the urinary nitrogen and consequently heat production may be underestimated.

The difference in excreted C, between HP and LP fed pigs, has implications for GHG production from manure; if quantitatively converted to CH_4, it would amount to 7,821 g/d of CO_2-equivalents. Such a reduction is attributable to the effect of diet manipulation on the pigs themselves and does take into account for any additional effects on GHG emissions related to manure that will contain less N and fermentable C. The CH_4 resulting from pig production also depend on the transformation of excreted N into N_2O. If quantitatively transformed, 19,400 g/d CO_2-equivalents could be produced. However, when aerating manure 5 to 30% of the N in manure may be lost as N_2O (Béline et al., 1999). Assuming these rates of N_2O loss leads to a two-fold difference in CO_2-equivalents of 3,919 (SE 107) g/d vs 8,365 (SE 213) g/d, it will affect data interpretation: the greater rate shows a significant diet effect, but the lower does not.

However, reducing dietary protein contents reduced the production of CO_2-equivalents even at the lower rate of conversion. Lowering dietary protein maintained animal performance; reduced GHG emissions by pig; reduced N and C excretion, and shows potential for reduced GHG emissions from excreta.

The results from Experiment 3 indicate that diets formulated exclusively on grains (barley) supplemented with appropriate amino acids can be fed to growing-finishing pigs. Feeding VLP diet maintained animal performance, reduced N excretion, reduced heat production (indicator of improved energy utilization). Also, such dietary manipulation can reduce GHG production by pigs. The core methodology is based on a combination of nitrogen and energy balances with indirect calorimetry which makes it possible to estimate protein, fat and energy retention and mobilization in the intact whole body (*in vivo*), total heat production and net substrate oxidation, as well as to calculate energy transfer between protein, carbohydrate and fat at the whole body level.

Reducing crude protein concentration of the diet of growing-finishing pigs with amino acid supplementation can markedly reduce nitrogen excretion. Swine producers looking for alternatives to reduce the amount of nitrogen excreted from swine should consider the use of very-reduced protein, amino acid supplemented diets. The public concern related

to the potential for water and air pollution from swine facilities demands that alternative management practices be developed. Dietary manipulation offers a potential method to reduce nutrient excretion by swine. Results from our experiments suggest that lowering crude protein by 6 percentage units with addition of amino acid can reduce total nitrogen excretion.

There is the possibility that pork producers could sell their reductions in GHG emissions as GHG credits to organizations that are not able to reduce their own emissions adequately. Carbon credits are valued at $15 – 60 per tonne (Ball and Moehn, 2003). A quantitative model describing the concomitant relationships between nutrient oxidation, protein retention and liponeogenesis from carbohydrates for pigs and poultry (and other animal species) could be evolved. Data from the gas exchange and respiration quotients could be used in modeling and evaluating ventilation rates in livestock buildings. Direct measurements of effectiveness of reduced dietary protein concentration on CO_2 emissions and possible effects of a dietary protein intake on CH_4 emissions are unknown.

Reducing dietary protein lowered nitrogen excretion and increased N retention (Experiment 4). Lowering dietary phosphorus reduced daily gain, but did not affect N retention. There was a reduction of energy (fat) retention associated with phosphorus reduction. Overall, the reduction of dietary phosphorus content impaired energy metabolism as shown by an increased heat production. Negative effects of low phosphorus (on daily gain, DE, ME and NE) were reversed by phytase addition to diets. Thus, reducing dietary phosphorus may reduce energetic efficiency. Individually, phytase and xylanase addition reversed growth depression caused by phosphorus deficiency and minimized phosphorus excretion. Adding phytase to low protein diets resulted in greatest net energy for the diet and highest energy retention. Such effects occur in intermediary metabolism and not during digestion. Optimizing dietary energy utilization can be achieved by reducing dietary protein, supplementing crystalline amino acids, and reducing dietary phosphorus in conjunction with addition with addition of phytase. The combined phytase-xylanase was efficient in reducing excretion and increasing P retention in growing pigs fed wheat-based diet. Phytase supplementation improved calcium and

phosphorus digestibility, while xylanase improved calcium and energy digestibility, but neither affected pig performance. Furthermore, there were no synergistic effects of phytase and xylanase on nutrient digestibility and pig performance.

Potentially, the best diets to minimize the environmental concerns regarding the excretion of large quantities of phosphorus in effluent from intensive pig production operations will require the use of well formulated low protein amino acid supplemented diets without supplemental phosphate, routinely supplemented with phytase or xylanase. A combination of the above measures should result in best utilization of dietary energy.

By-products (wheat middlings) could become more valuable feed ingredient if the nutrients bound by arabinoxylans and phytate were made available for use by the pig through enzyme supplementation. Wheat middlings contain digestible nutrients and should thus be considered a feedstuff for swine. Xylanase and/or phytase will improve the nutritional value of wheat middlings for growing-finishing pigs.

7.1 Prospects for Future Research

An extensive review of the literature (Chapter 2) and the results from four experiments (Chapters 3 to 6) suggest that there are many factors and interactions among dietary components that influence the effect of reduced-protein AA-supplemented diets, low-phosphorus level, phytase and xylanase supplementation individually and in combination on the utilization of different nutrients and energy. Therefore, it is understandable to find different responses in terms of utilization of different nutrients and energy when reduced-protein AA-supplemented, low-phosphorus level, phytase and xylanase supplementation individually and in combination, is studied in one experiment of different diets. A key issue was how to predict these responses for different parameters with different diets based on the information from specific feed ingredients.

As was discussed in Chapter 2 (Literature review), dietary factors that govern or interact with the responses to phytase supplementation include the dietary contents of phytate, different minerals, vitamins (e.g., vitamin D), intrinsic phytase activity, and some anti-

nutritive factors. Clearly, the interactions among these dietary factors are a multifaceted subject which merit further studies. For further research, opportunities exist to identify these nutritional factors and interactions, and quantify their effects on the magnitude of responses to phytase. Following, linear or non-linear computer models should be developed to accurately predict animal responses to phytase for different parameters with different diets. The data generated from the computer models would not only encourage the swine industry to increase the application of phytase, but also assist feed manufacturers to more accurately formulate diets that will precisely match animal requirements for maintenance and growth with dietary supply.

The benefit of dietary supplementation of phytase in reducing P output from the swine industry has been obvious. However, the effect of phytase supplementation on the utilization of other nutrients (other than P) is not confirmed, in light of current knowledge. From available information, it has been difficult to exceed P bioavailabilities of 60 to 70% in feed ingredients of plant origin, even if phytase is supplemented at a very high rate. Current use of commercially available phytase as a routine feed additive is also limited by inactivation of phytase at the high temperature (65 to 80°C) required for steam-pelleting (Lei and Stahl, 2001), and by loss of activity during long-term storage/transport at ambient temperatures (Lei and Stahl, 2001). Therefore, for the enzyme industry there are also opportunities that exist to improve the efficacy or quality of their phytase products.

Opportunities exist to design experiments to investigate the impact of reduced dietary CP content on reducing excretion of N in manure and its volatilization as NH_3, which is considered as an "indirect" GHG and on N_2O emissions from manure during storage and field application. There should be research to look at fiber, in terms of digestible vs. non-digestible or fermentable vs. non-fermentable. Also, in collaboration with agricultural engineers odor research work could be intensified to identify the specific amino acids that had greater potential to elevate odor from swine facilities due to their level of inclusion in diets. This research would be vital because of the importance of odor in the perception of swine production by the public, this area of research warrants further investigation.

Research efforts towards developing the net energy (NE) system for swine diet formulation by the Canadian feed industry should be intensified. Adoption of the NE system will result in assignment of a lower economic value to feedstuffs with indigestible and fermentable fiber. This will also reduce the potential for methane production from stored manure. The reduction of CP contents increases the energy available for tissue deposition (Le Bellego et al., 2001). This agrees with the NE system (Noblet et al., 1994) which assumes that proteins are less efficiently used than starch (60 vs. 80% for ME). However, the NE system was established with diets having higher CP levels (17.3% on average) than those currently used for growing pigs. Level of feed and protein intake may change the rate of protein and lipid deposition and thus alter diet NE. Protein and lipid deposition, have differing energetic efficiencies. A change in the ratio of protein to lipid deposition caused by feed intake and protein concentration may alter the dietary energy content can be precisely calculated from C-N balance, and heat production (HE) predicted (estimated) from digestible nutrient contents.

Limited research has been conducted to study the effects of reducing particle size in combination with supplementation of either particle size in combination with supplementation of either phytase or carbohydrase on nutrient digestibility and N and P excretion patterns in grower pigs. Also, more research is needed to determine the effect of phytase-xylanase addition in diets without the inclusion of trace mineral premixes in swine diets. The full benefit of enzyme addition (especially phytase) could be realized if it is cheaply available than the cost of sources of mineral premixes and can be used exclusively without inclusion of the mineral premixes.

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